THE EFFECTS OF DEICING AGENTS ON THE AUTOTROPHIC AND HETEROTROPHIC COMMUNITIES OF LAKE TAHOE

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16. ABSTRACT

From December 1980 to July 1981, 12 bioassays were performed to determine the potential impacts of four deicing agents on the natural communities of algae and bacteria in Lake Tahoe. Kiln-dried salt and sand were shown to have no significant impact on algal or bacterial growth. Processed salt was observed to stimulate algal growth and inhibit bacterial growth during certain periods of the year. The adverse effects of processed salt may be attributable to the ferrocyanide "anticaking" compound applied to this salt. Cinder was shown to stimulate either algal or bacterial growth at certain times of the year. Based on these findings the recommendation was made to continue the use of kiln-dried salt and sand abrasive, and to discourage the use of processed salt and cinder. Stream sampling indicated that chloride levels in tributaries along the west shore of Lake Tahoe were extremely low, whereas those of tributaries traversing populated areas or crossing the highway several times were slightly elevated. Lake Tahoe periphyton growth was shown to be unaffected by either processed or kiln-dried salt at low concentrations. Further study of the effects of these salts on periphyton N_2-fixation is required before a final assessment of their impact can be made.

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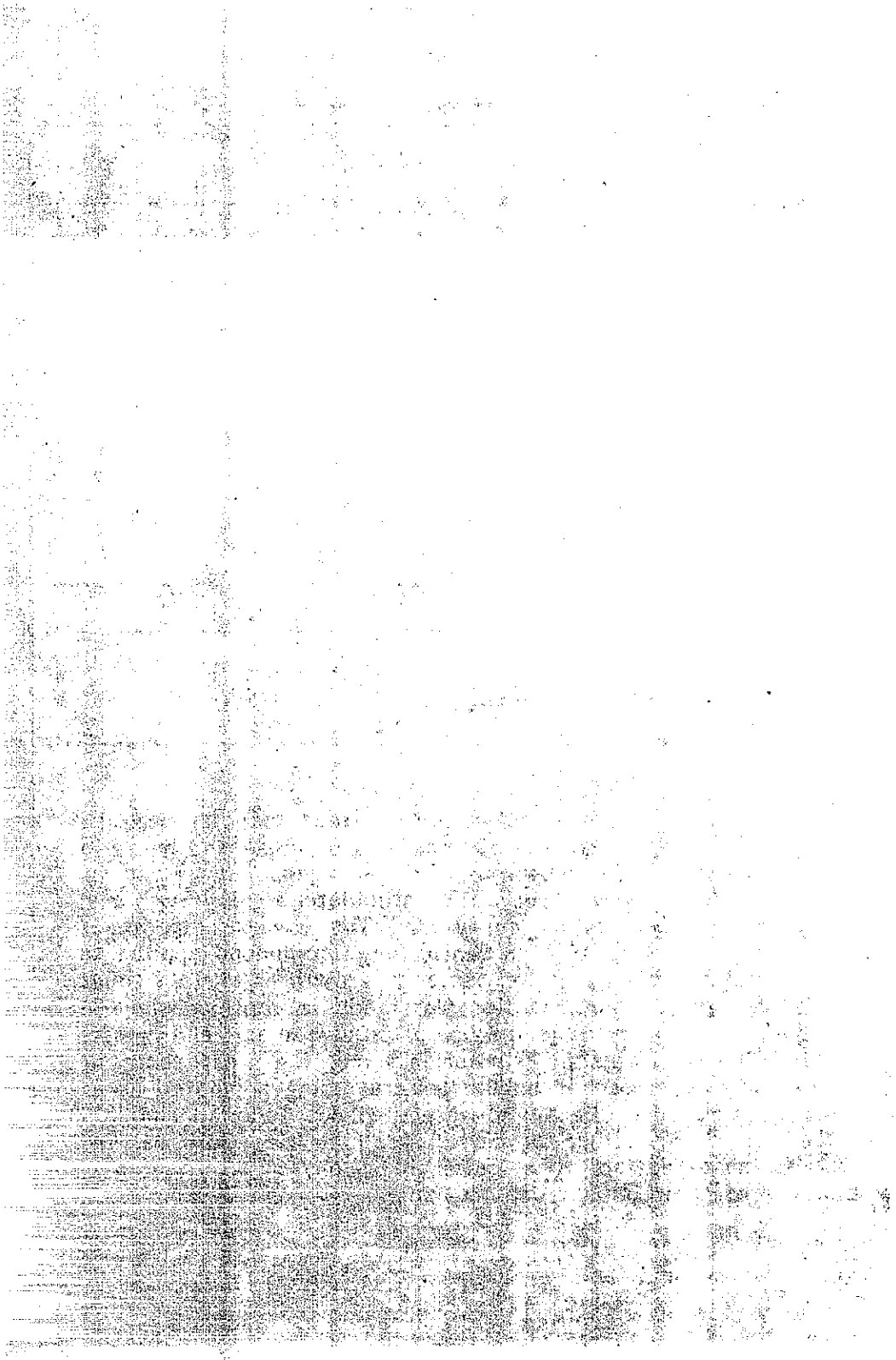

THE EFFECTS OF DEICING AGENTS ON THE AUTOTROPHIC AND HETEROTROPHIC COMMUNITIES OF LAKE TAHOE

Contract Number E13563; Performing Organization Report No. 19703-604193. A study done in cooperation with the Caltrans Office of Transportation Laboratory under the research project titled "Microcosm Study of the Effects of Deicing Salt on Aquatic Ecosystems. The total expenditure made under this contract did not exceed $36,026.

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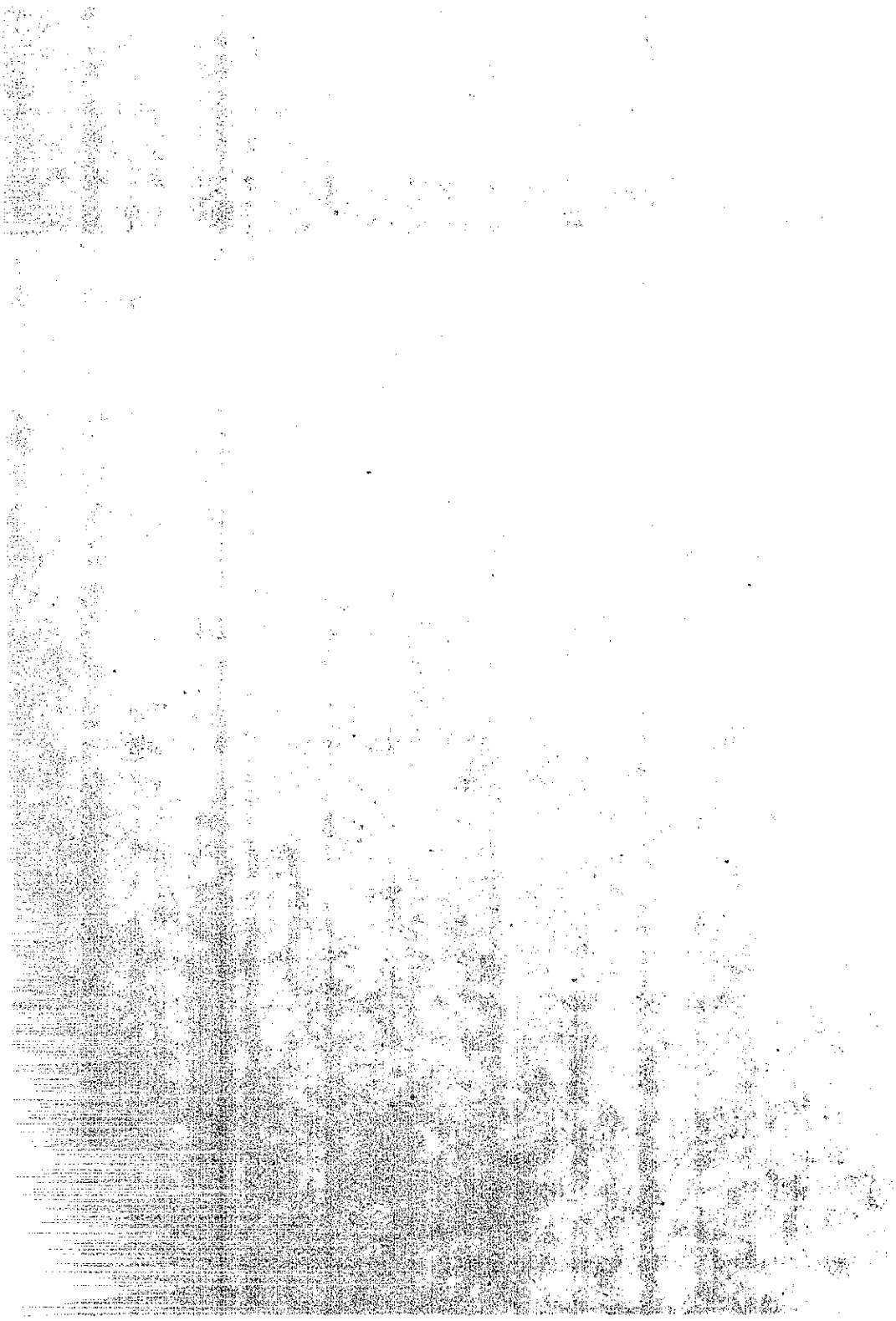

I. INTRODUCTION

HISTORICAL BACKGROUND

In 1974-75, the California Department of Transportation sponsored a twenty month comprehensive study by Ecological Research Associates to investigate the environmental effects of deicing agents on aquatic systems in the Lake Tahoe basin and vicinity. Several important findings of that study have served to aid Caltrans in its effort to minimize the impacts of deicing agents on the environment. Briefly, this study showed:

1) Lakes with Interstate-80 within their watershed exhibited chloride enrichment relative to other California lakes; the density profile in one roadside pond (under ice and snow cover) was found to be temporarily but strongly affected by salt-laden roadside runoff.

2) Stream chloride levels were found to increase and fluctuate throughout the period of salt applications.

3) Numerous deicing salt contaminants of possibly stimulatory or inhibitory nature to plankton were found. Deicing salts from different suppliers were shown to have different degrees of contamination.

4) Bioassay experiments showed inhibition of Donner Lake bacterial metabolism by three deicing salts used by Caltrans at the time of the study. Algal metabolism was not altered by any of the eight deicing salt samples assayed.

Goldman and Hoffman (1975) concluded that, although road salting resulted in detectable chloride enrichment in many Sierran lakes, there was no apparent indication of its detrimental effect on the endemic biota. However, it should be noted that only one bioassay was performed using the natural community of

Lake Tahoe algae and bacteria. The majority of the 1974-75 research used Donner Lake water, which has five times the chloride content (11 mg/l) of Lake Tahoe and greater algal productivity.

Prior to this initial study on the aquatic impacts of deicing salts, one study had been completed and another initiated regarding the impacts of deicing salts on roadside vegetation in the Tahoe area. The U.S. Department of Agriculture Forest Service conducted a study in 1973 to evaluate the cause and distribution of damage to roadside conifers, species-specific effects, and the gradient of damage with distance from the highway (Scharpf and Srago, 1974). They concluded that salt-laden highway runoff was a major cause of tree damage and death in the Tahoe basin at the then-current levels of application. However, this study did not attempt to correlate the extent of damage to the amount of salt applied in different sections of the highways, nor did it take into account other possible causes of plant damage.

Therefore, in late 1973 Caltrans and the Federal Highway Administration financed a five year study conducted by the Department of Environmental Horticulture of the University of California, Davis (Leiser et al., 1980), to address these and additional questions surrounding highway deicing operations and impacts on roadside vegetation. The results of the Leiser study confirmed highway deicing salt to be a cause of damage in conifers but also indicated that beetles were another important cause of tree damage both near and away from highway corridors. Relative salt tolerances were determined for each of the four common conifers and, for three of these, critical levels of soil salt per highway salt application were estimated.

Since the initial studies in 1973 and 1974-75, the California Department of Transportation has monitored the statewide use of salts closely and attempted to reduce their usage whenever and wherever possible. In the Tahoe basin, the

use of salt has been primarily limited to kiln-dried salt which was identified in the previous study to have little impact on the aquatic biota. A "no salt" test section on Highway 89 near Emerald Bay has also been established in order to evaluate the impacts of not using salt on: traffic safety, number and duration of road closures, and the recovery of previously salt-damaged vegetation. The results of this study will be used for comparison with similar sections of salted roadway to determine the feasibility of eliminating salting altogether in some environmentally sensitive areas.

The renowned water clarity and aesthetic appeal of Lake Tahoe are largely the result of extremely low rates of nutrient loading from the adjacent watershed. Numerous studies have indicated that, although the lake remains ultraoligotrophic, it has been undergoing accelerated eutrophication for the past two decades as a result of increased loading of sediments and dissolved nutrients from disturbed watersheds (Goldman, 1974, 1981; Leonard et al., 1979; Elder et al., 1976). In particular, nitrogen and iron, and less frequently phosphorus (all at part per billion levels), have been shown to significantly stimulate phytoplankton production. Therefore, inputs of nutrients or other compounds above natural levels are undesirable in the Lake Tahoe basin.

The present study was conducted in the winter, spring, and early summer of 1980-81 and was designed to determine the potential impacts of currently used deicing agents (salts and abrasives) on the natural communities of algae and bacteria in Lake Tahoe surface waters. Of special interest was the impact of processed salt, relative to the more impurity-free kiln-dried salt, since it has been proposed for use on Highway 267 between Truckee and Kings Beach. An additional goal was to accumulate data supplemental to that reported by Goldman and Hoffman (1975) regarding present levels of salt in Tahoe basin waters. Importantly, the duration of the study allowed us to analyze the response of

the aquatic microbial biota during varied lake conditions including mid-winter, spring runoff-influenced, and early summer periods. The composition of the microbial communities in lakes typically changes with the seasons and reflects changes in the condition of the lake. Because winter through early summer is the time during which the lake is most likely to be influenced by saline input from deicing salt applications, it was desirable to assay the particular agents' effects on biota representative of each of these seasons.

The results of the present study, when integrated with the results from previous basin road salt influence studies, should further the development of a road salt use management strategy which minimizes adverse environmental impacts.

EFFECTS OF SALTS ON ALGAE AND BACTERIA

Although numerous studies have involved assessing the environmental impacts of deicing agents (see Hanes et al., 1970), very few have examined their effect on aquatic microbial communities. However, an extensive microbiological literature does exist regarding the effects of various salts on many aspects of algal and bacterial metabolism. The following is a brief summary of some of the more important findings pertinent to this study:

1) Sodium, which constitutes approximately 39 percent of the rock salt used in deicing, is an essential micronutrient for many algae. Anabaena cylindrica and certain other freshwater blue-green algae require appreciable concentrations of this element (Allen, 1952, 1955). It is thought to be required by nitrogen-fixing blue-green algae for the transformation of N_2 into ammonia (Brownell and Nicholas, 1967).

2) Chloride, which constitutes approximately 60 percent of rock salt, is required by all photosynthesizing algae as a micronutrient. It appears to play a role in several important photosynthetic reactions (Stewart, 1974).

3) Bacteria appear to vary in their specific requirements for sodium and chloride. In most nonhalophilic (high salinity intolerant) bacteria a sodium requirement has not been demonstrated, while bacteria of marine origin and halophilic bacteria have a definite requirement for sodium (Stanier et al., 1976).

4) In both algae and bacteria, sodium and chloride may have important osmoregulatory roles, although in bacteria they are probably of lesser importance in this regard than potassium (Stanier et al., 1976). While small amounts of sodium and chloride are required by many microorganisms for growth, excessive amounts of these ions may restrict growth and damage cells. These detrimental effects can often be ascribed to osmotic effects. Osmosis is the mechanism by which water balance is maintained in these organisms. Both algae and bacteria tend to maintain internal ion concentrations above that of the surrounding medium creating conditions favorable to diffusion of water inward. Sudden changes in external salinity (such as might occur with influx of deicing salts into natural bodies of water) increase the external concentration of ions above internal levels, causing water to diffuse out of the cells. In response to such conditions, microorganisms attempt to regain water balance by accumulating ions internally. It is both the loss of water and the increased accumulation of ions within the cells which may have detrimental impacts on these microorganisms.

5) The algae exhibit varied responses to osmotic effects: stimulation or inhibition of respiration as well as inhibition of photosynthesis may occur (Stewart, 1974); certain algae (e.g. Chlorella) may respond with loss of organic phosphates to the external medium (Antonyan and Pinevich, 1966); the same algae may also respond with inhibition of daughter cell formation and increases in cell biomass (Soeder et al., 1967).

7) Varied effects of osmotic stress on the bacteria may also be expected, but not necessarily at the same concentrations which affect the algae. Unlike most algae, bacteria are enclosed by a rigid cell wall capable of withstanding considerable internal water pressure. Thus, they may maintain internal ion concentrations well above that of the medium without risk of cell lysis (bursting) due to diffusion of water into the cell. Because most algae do not have such a rigid outer wall, they must maintain a smaller difference between internal and external ion concentrations. Consequently, bacteria may be less immediately affected by small changes in salinity than algae.

Road salts also contribute various macronutrient, micronutrient and trace element contaminants along with sodium and chloride to melt waters (Goldman and Hoffman, 1975). These contaminants may similarly elicit varied bacterial and algal metabolic responses. Iron has been shown to be a contaminant in several salts including the processed salt assayed in this study. It should be noted that iron is an essential component of the enzymes nitrite reductase, nitrate reductase and nitrogenase, all of which are fundamental to cellular nitrogen assimilation—which has been shown to be closely linked to algal growth in Lake Tahoe (Stewart, 1976; Goldman, 1974, 1978, 1981).

II. CONCLUSIONS AND RECOMMENDATIONS

1. One of the desired goals of this study was to determine the impacts of processed salt on Lake Tahoe aquatic bacterial and algal communities, as its use has been proposed along Highway 267 between Truckee and Kings Beach. The results of sensitive ^{14}C-uptake bioassays have indicated that this salt (at realistic concentrations) will, in fact, stimulate algal growth and inhibit bacterial growth at certain times of the year. Stimulation of algal growth was observed at concentrations of 10 mg/l Cl^- and inhibition of bacterial growth at concentrations possibly as low as 1 mg/l Cl^-. With both stimulatory and inhibitory effects (both of which may be considered adverse) attributable to this salt and since kiln-dried salt, which has no effect at similar concentrations, is readily available, the use of processed salt within the Tahoe basin would not be recommended. Its use should be particularly discouraged in areas where road "contact" with a stream is extensive. Goldman and Hoffman (1975) attributed the extensive contact of Highway 50 with the Upper Truckee River to be largely responsible for the elevated chloride concentrations measured in the river. A somewhat similar situation exists along Highway 267, as two basin streams parallel the highway in close proximity for considerable distances. In Figure 1 it can be seen that Snow Creek runs parallel to and ~300-500 yards downslope of the highway, for a distance of approximately one mile, just below Brockway Summit. As a consequence, all highway runoff along this section drains toward, and is eventually collected in, Snow Creek. Two seasonal drainages are also noted to "contact" the highway within the area proposed for processed salt use, and eventually join with Snow Creek. Griff Creek runs nearly adjacent

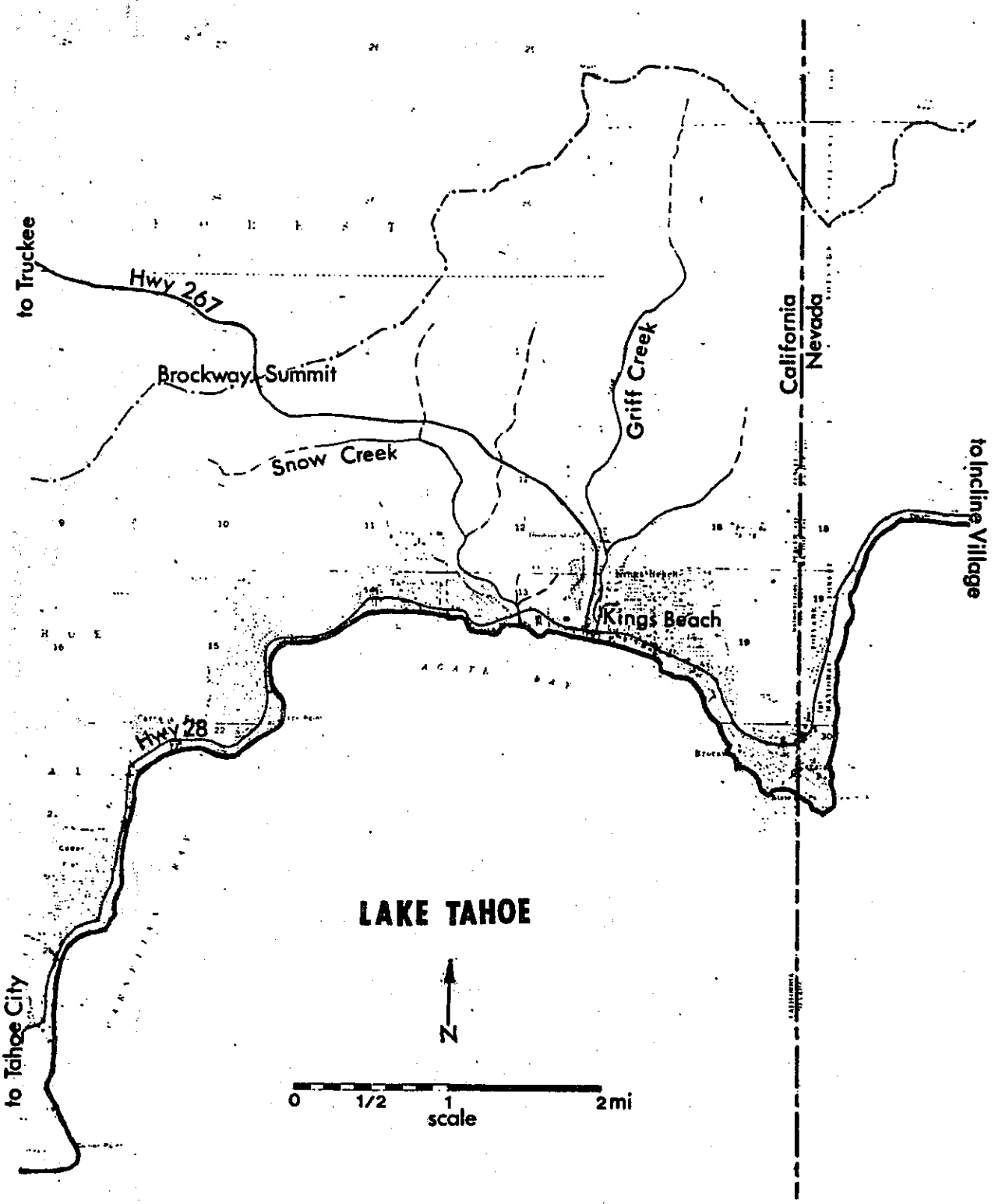

Figure 1. Highway 267 in the proximity of Snow and Griff creeks. (Map after Jorgensen et al., 1978)

to the highway for approximately 1/2 mile near Kings Beach, and would be expected to receive direct street runoff.

2. The continued use of kiln-dried salt for deicing operations within the Lake Tahoe basin is preferred. At a concentration equivalent to the highest sustained chloride levels in a basin tributary (10 mg/l), this salt had no significant effect on phytoplankton, bacterial, or periphyton growth.

3. The inhibitory and stimulatory effects caused by processed salt are thought to be the result of chemicals derived from the ferrocyanide "anti-caking agent" applied to this salt. Ferrocyanide, $Fe(CN)_6$, decomposes (rapidly in the presence of sunlight) to release iron and cyanide. Iron has been previously indicated by Goldman (1974, 1978, 1981) to be an important nutrient limiting Lake Tahoe algal growth. It is likely that the algal stimulation caused by processed salt was due to its addition to the lake water during a time in which iron deficiency was severe in algal populations. The inhibitory effect of this salt on the bacteria may have been the result of a sensitivity to the cyanide component.

4. Of the two deicing abrasives studied, sand appears to be more desirable for use in the basin, as it did not affect algal or bacterial growth. Use of cinder is less desirable as it proved to be stimulatory to both algal and bacterial growth. Chemical analyses suggest that the soluble phosphorus content of the cinder may have been responsible for the stimulatory effects seen. Selected cation analyses indicated that the cinder also contributed more Na^+ and K^+ to solution than did sand.

5. Levels of kiln-dried and processed salt above 50 mg/l Cl^- may inhibit algal growth at certain times of the year. Periods of increased tolerance to elevated salinities appear to correlate with periods of increased nutrient

availability. The nutrients contributed by processed salt appear to counterbalance, to some extent, the inhibitory effects of high Cl^- levels.

6. Neither kiln-dried salt nor processed salt were shown to affect periphyton growth at a concentration of 10 mg/l Cl^-.

7. Bioassays conducted using tributary and snow samples having different levels of chloride contamination produced results which did not always correlate with chloride concentration. Since non-stimulatory kiln-dried salt is currently most widely used in the basin and expected to be the contaminating salt in these samples, stimulatory effects were probably not due to the road salt. The observed stimulation was more likely the result of other road-derived contaminants including cinder.

8. Tahoe's tributaries along the west shore of the lake are very low in chloride. Blackwood, Eagle, General, Meeks, Tallac and Ward creeks contained levels ranging from 0.146 to 0.606 mg Cl^-/l. These chloride levels appear to be a direct reflection of the chloride content of precipitation and not of road salting.

9. No consistent pattern of chloride increase below highways was observed for those tributaries sampled above and below major basin highways. This was true even at the ppb level of analysis. More intensive sampling of basin tributaries above and below the highway, during and immediately following deicing salt applications, must be performed before conclusions may be drawn regarding inputs from basin highways. As our results show, the premise that where stream contact with the highway is minimal, roadsalt impact is minimal--appears to be valid.

10. In many areas around the lake, tributaries may not constitute the major source of chloride input. Given the frequent close proximity of basin

highways to the perimeter of the lake, it is possible that direct surface runoff and groundwater contribute greater proportions of chloride.

III. METHODS

Deicing salts and abrasives were obtained from the California Department of Transportation maintenance stations at Echo Summit (kiln-dried salt, cinder) and Truckee (processed salt, sand) for microbial assays and for determination of macronutrient content. Macronutrient analyses were performed on diluted salt solutions or abrasive leachate solutions using the following methods: Nitrate-N by hydrazine reduction of nitrate to nitrite followed by colorimetric analysis (Strickland and Parsons, 1968); ammonium-N by the blue indophenol reaction with the removal of interference by complexation with citrate (Solorzano, 1969); biologically available iron by Ferrozine colorimetry (Stookey, 1970); total-P by the ascorbic acid-phosphomolybdate method after acid hydrolysis (Strickland and Parsons, 1968; A.P.H.A., 1971). Specific cation analysis (Na^+, K^+, Ca^{++}, Mg^{++}) were also performed on sand and cinder leachate solutions by atomic absorption using a Beckman Atomic Absorption system.

Tributary and snow samples were collected from areas of varied road salt influence (see Figure 2) and stored frozen in polyethylene bottles. Selected subsamples were assayed for their effects on Lake Tahoe phytoplankton as described below, and all samples were analyzed for chloride content using a Graphic Controls Ultrasensitive chloride probe (limit of detection 0.050 mg/l Cl^-). Chloride analyses were also performed on samples taken at several day intervals from seven basin tributaries during the spring snowmelt period in 1981. These stream samples were collected by the Tahoe Research Group (TRG) and represent some of the major sources of nutrients to the lake. Chloride content was determined by the TRG using the sensitive (limit of detection 0.025 mg/l Cl^-) colorimetric assay developed by Florence et al. (1971).

Regular sampling to determine fluxes of deicing salts in basin tributaries was not attempted. Goldman and Hoffman (1975) have indicated these fluxes

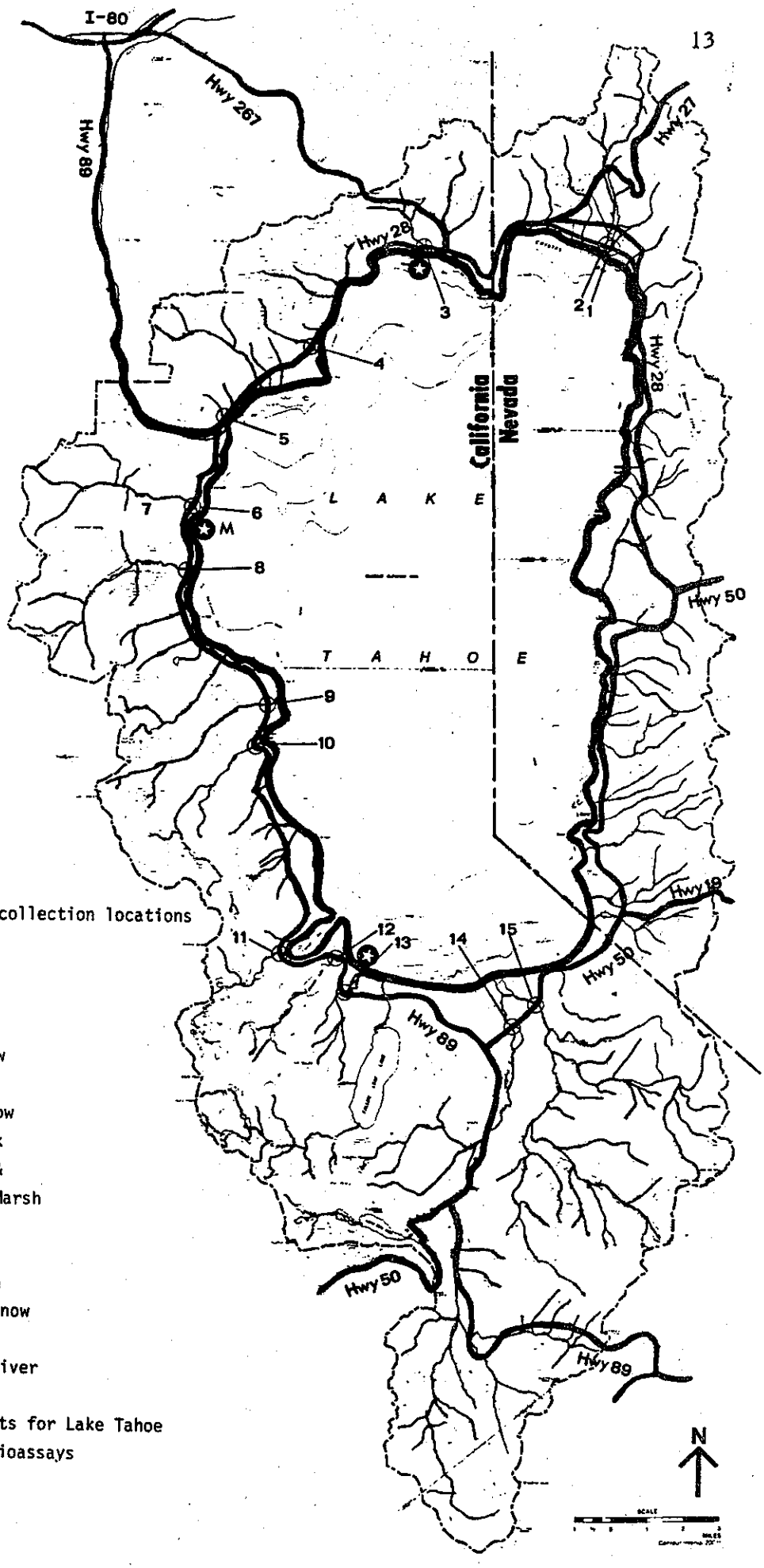

Figure 2. Sample collection locations

1- Incline Creek
2- Third Creek
3- Snow Creek
4- Dollar Creek
5- Tahoe City Snow
6- Ward Creek
7- Ward Valley Snow
8- Blackwood Creek
9- General Creek &
General Creek Marsh
10- Meeks Creek
11- Eagle Creek
12- Cascade Creek &
Cascade Creek Snow
13- Tallac Creek
14- Upper Truckee River
15- Trout Creek
● = Collection points for Lake Tahoe water used in bioassays
M = Main station

to be of brief duration, and a relatively intense sampling regime immediately following road salt applications would be needed to accurately estimate total inputs. However, such sampling would require personnel located within the basin, closely attuned to storm activity and subsequent deicing operations.

Phytoplankton enumeration was performed on selected bioassay subsamples using Utermohl settling techniques described in Nauwerck (1963).

Algal growth bioassays were performed on Lake Tahoe littoral zone water using the $^{14}CO_2$ uptake method previously described by Goldman (1967) and Goldman et al. (1969). Water containing natural populations of phytoplankton was collected from the lake's surface (see Figure 2 for locations) using 20 liter opaque carboys and immediately transported to the lab at Davis while being maintained at low temperature and illumination. Assays were generally initiated within 24 hours of sample collection. The lake sample was filtered through 80 μ nitex netting (to remove larger zooplankton) and then innoculated with a small volume of $Na_2\ ^{14}CO_3$ (final concentration ~20 $\mu Ci l^{-1}$). The water was then thoroughly mixed and distributed into 500 ml Erlenmeyer flasks to which small volumes of the various salt solutions had been added, and final volumes were adjusted to 500 ml in each flask. Deicing salt enrichments generally consisted of 0.5 or 5.0 ml additions of saline solutions as ppm Cl^-. Sand and cinder leachate enrichments were prepared by placing 5 grams of sand or cinder in 500 ml of quartz double distilled water and allowing the material to leach for either 24 hours at room temperature ~23°C (bioassay 4) or 48 hours at 12°C with constant agitation (bioassays 8 and 9). The leachate solutions were then filtered through Whatman GF/C filters and the filtrate added in 0.5 or 5.0 ml quantities to treatment flasks. Stream/snowmelt water enrichments consisted of 50 ml additions of GF/C filtered sample (10% by volume). The flasks were

incubated in a psychrothermic incubator at temperatures (~5°C in winter; ~14°C in late spring) and diel illumination approximating ambient lake surface values. Fifty milliliter subsamples were removed from the flasks at two day intervals and filtered onto 0.45 µm Millipore membrane filters. Filters were then air dried and radioactivity determined using a Geiger-Müeller gas proportioning counter.

Bacterial bioassays were performed using methods similar to those described by Paerl and Goldman (1972) and Goldman and Hoffman (1975). The method is based on the fact that microbial uptake of acetate at low concentrations (<100 µg/l) is rapid and almost solely attributable to bacteria (Wright and Hobbie, 1965). Briefly, lake water containing natural phytoplankton and bacteria was enriched with salt and ^{14}C-labeled bicarbonate added as described above for algal assays. At two day intervals, two aliquots were removed from each incubation flask. The first (50 ml) was immediately fixed with 0.1 ml of Lugol's solution to inhibit further metabolic activity and then filtered onto 0.45 µ Millipore filters. The second (100 ml) was innoculated with radioactive acetate (1.0 ml of 2.0 µ Ci m^{-4} ^{14}C-acetate; final carrier concentration of ~23 µg acetate l^{-1}), and then incubated with slight agitation (@ 40 rpm), in the dark for 1-1.5 hours. Incubations were terminated using Lugol's solution, as above. Fifty milliliters were then filtered (0.45 µ), and successively rinsed with 10 ml portions of 0.1 N sodium acetate and distilled water to liberate absorbed/adsorbed ^{14}C-acetate. Radioactivity was then determined as for autotrophic assays. Using this method it is possible to obtain simultaneous estimates of autotrophy and heterotrophy for a given water sample. Heterotrophic uptake is estimated by subtracting the radioactivity attributable to autotrophic ^{14}C bicarbonate uptake (the first sample) from that attributable to uptake of both ^{14}C bicarbonate and $2^{-14}C$ acetate (the second sample).

It should be noted that the preliminary methods used in bioassays #5 and #7 were slightly different from the final method described above. In bioassay #5, we did not add ^{14}C-bicarbonate at the start. ^{14}C-acetate uptake was determined as above and this method had the advantage that some variability among replicates due to slightly different rates of uptake of ^{14}C-bicarbonate was removed. The disadvantage was that a simultaneous autotrophic uptake rate was not determined. Also, in bioassay #5, 2.5 ml/50 ml sample of 2N H_2SO_4 was used as the metabolic inhibitor. Due to the possibility of cell lysis upon acidification, a supercooled ($\sim$-3°C) saline ice bath was used in bioassay #7 to inhibit metabolic activity. In both bioassays #5 and #7 a smaller pore size filter (0.22 µ HA Millipore) was used to assure retention of the smallest bacterial cells. However, this treatment greatly increased filtration times, producing a larger uncertainty in the actual incubation time. Interestingly, the radioactivity retained on this filter was anomalously lower. Self-absorption, lysis of cells or some other aspect of the procedure may be the cause. It was decided that 0.45 µm would provide sufficient retention and substantially decrease the time required for filtration, thus increasing overall experimental precision.

For statistical analysis of bioassay data, a protected F-test was chosen to compare bioassay treatment means. It is considered to determine significance of treatment differences with stringency balanced between the extremes of leniency and ultra-conservatism. In a protected F-test treatment means are compared only if a significant difference among means is detected by a preliminary analysis of variance (AOV) (Snedecor and Cochran, 1967). In this study treatment contrasts were limited to treatment vs. the control. Although levels of significance for treatment contrasts may occasionally exceed the level of significance of the preliminary F-value, it should be recognized that differences

between treatment means cannot truly be considered "more significant" than the level of significance indicated by the AOV F-value.

IV. RESULTS AND DISCUSSION

DEICING SALT APPLICATIONS

A summary of deicing salt applications for the winter 1980-81 indicated 412 total tons were applied to state maintained roads within the basin. Table 1 presents the detailed schedule of those applications according to highway section. In addition to the state deicing operations, it should be noted that local entities (usually the counties) are responsible for road salt and abrasive additions to non-state highways. Contributions to road salt inputs by these agencies, as well as those by the State of Nevada, should be studied in the future. The data for 1980-81 indicate a substantial decrease from the 1155 tons of salt applied on the California side of the basin in 1974-75 (Goldman and Hoffman, 1975). Water year 1980-81 was unusually dry and the lower application rates thus are a reflection of both subnormal precipitation and the concerted effort on the part of Caltrans to reduce salt usage.

CHLORIDE LEVELS IN LAKE TAHOE BASIN WATERS

Basin tributaries were sampled throughout the study to meet the following objectives: (1) using more sensitive methods for chloride analysis to determine small (microgram/liter) changes in chloride concentration above and below lightly salted basin highways for use as indicators of loading patterns similar to those readily observed for more heavily salted roads; (2) to determine the effects of road salt-laden stream and snow samples on indigenous Lake Tahoe phytoplankton using bioassay techniques; (3) to obtain a general comparison of the chloride levels in several basin tributaries.

In addition to our sampling program, routine sampling of water from seven basin tributaries was performed by the Tahoe Research Group during the spring snowmelt period and the samples made available for our analysis. These

Table 1

DEICING SALT APPLICATIONS: WINTER 1980-1981

Lake Tahoe Basin (Total 412.2 tons)

County Line (Tahoma) to Tahoe City
Pla 89 P.M. 0.0 - Pla 89 P.M. 8.5 (17.0 lane miles)

Total tons = 74.9
Tons/Lane Mile = 4.4

N. Boundary "No Salt Test Sect." to Tahoma
E.D. 89 P.M. 19.2 - E.D. 89 P.M. 27.4 (17.4 lane miles)

Total tons = 34.0
Tons/Lane Mile = 2.0

Tahoe City to State Line
Pla 28 P.M. 0.1 - Pla 28 P.M. 11.0 (30 lane miles)

Total tons = 87.2
Tons/Lane Mile = 2.9

Brockway Summit to Kings Beach (6 lane miles)

Total tons = 39.8*
Tons/Lane Mile = 6.6*

Echo Summit to Myers
E.D. 50 P.M. 66.88 - E.D. 50 P.M. 70.4 (7.48 lane miles)

Total tons = 49.3*
Tons/Lane Mile = 6.6*

Lower Myers Grade to State Line
E.D. 50 P.M. 70.40 - E.D. 50 P.M. 80.4 (39.80 lane miles)

Total tons = 73.0
Tons/Lane Mile = 1.83

County Lane to S. Boundary "No Salt Test Sect."
E.D. 89 P.M. 0.00 - E.D. 89 P.M. 9.9 (23.40 lane miles)

Total tons = 54.0
Tons/Lane Mile = 2.3

"No Salt Test Sect."
E.D. 89 P.M. 9.90 - E.D. 89 P.M. 19.2 (18.60 lane miles)

Total tons = 0
Tons/Lane Mile = 0

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* estimated

tributaries included Snow, Ward, General, Trout, and Third Creeks, and the Upper Truckee River—all very important to the nutrient budget of the lake (Leonard and Goldman, 1981). From these data it was hoped that trends in the chloride content of tributaries associated with the progression of the snowmelt would become apparent. In addition to stream samples, several snow samples were obtained from areas of varying road salt influence. These were analyzed for chloride content, and a few chosen for assay of their effects on lake phytoplankton.

Before proceeding to the results and discussion of the stream sampling, it should be mentioned that no effort was made to determine the exact fluxes of deicing salts into Lake Tahoe via basin tributaries. Goldman and Hoffman (1975), using a twice monthly sampling regime on four basin tributaries, were unable to detect any Cl^- fluxes in three of the four. Similar results were obtained using a daily sampling regime during one storm period of moderate snowfall. Streams which did show evidence of elevated Cl^- levels were found to exhibit maximal inputs shortly after the storms began. This suggests that salt influx might be of extremely brief duration, perhaps corresponding to the actual period of application and the time shortly thereafter.

The results of all Cl^- analyses are reported in Appendix E. It can be seen that Blackwood, Eagle, General, Meeks, Tallac and Ward creeks all exhibited very low Cl^- values (range: 0.146 mg/l - 0.606 mg/l) for the dates sampled. Each of these streams emerge from relatively undisturbed watersheds and contact the highway only briefly at a relatively short distance from the lake. Since there is minimal exposure to roadways subject to deicing, the chloride levels in these streams are most likely a direct reflection of chloride in the precipitation itself. Precipitation data collected in the Ward Valley for 1980 indicate Cl^- levels in rain and snow ranging from a minimum of 0.050 mg/l to a maximum of

0.656 mg/l (Leonard and Goldman, 1981). Both Ward and General Creeks were sampled throughout the study, but only in Ward was a slight decrease in Cl^- noted, which was associated with the seasonal change from winter to spring. Meeks Creek, which was sampled only twice, also had slightly lower Cl^- values later in the season. In general, these results indicate that Tahoe's tributaries along the west shore of the lake are very low in Cl^-. The levels are comparable to those reported in an undisturbed watershed not subject to deicing--i.e. Hubbard Brook, New Hampshire which has an average runoff Cl^- concentration of 0.540 mg/l (Likens et al., 1977).

Trout and Dollar Creeks exhibited Cl^- values somewhat higher than values of those previously discussed, possibly indicating increased road salt influence. Dollar Creek was at a very low flow when sampled in April compared to those tributaries previously discussed, suggesting that any road salt contamination derived from the highway was subject to less dilution. Trout Creek, which was sampled fairly regularly from March to late May, gave a mean Cl^- content of 0.675 mg/l with Cl^- concentrations appearing to decrease as the season progressed.

Incline Creek, Third Creek and Upper Truckee River were all sampled regularly during the spring runoff and exhibited means of 2.290 mg/l, 1.281 mg/l and 1.624 mg/l, respectively. Incline and Third Creeks, though located in Nevada, are included with the present data to show the increased Cl^- concentrations which might be typical of streams traversing large populated areas. The Upper Truckee River, which was sampled fairly extensively by Hoffman throughout 1974-75, gave comparable data in the present study with values fluctuating around 1.6 mg/l.

A natural drainage traversing a residential area in Tahoma, and Snow Creek at Tahoe Vista, gave the highest Cl^- concentrations observed in waters which were not primarily due to street runoff. The high Cl^- content of the

Tahoma sample may be a reflection of county maintained deicing activity on residential streets. The elevated Cl^- levels in Snow Creek are probably derived from an adjacent snow disposal site. In a study of this particular disposal site, which receives snow from the business areas of Kings Beach, Tahoe Vista and Carnelian Bay, Foster (1971) determined an average snow Cl^- concentration of 54 mg/l. This is very close to the 69 mg/l Cl^- value reported by Goldman and Hoffman (1975) for obviously contaminated roadside snow. A decrease observed in Snow Creek Cl^- from 10.290 mg/l in mid-May to 2.941 mg/l in July seems to support the idea that the Cl^- was derived directly from the disposal site, as all snow had long since melted by July.

No consistent pattern of Cl^- increase below the highways was observed for those tributaries sampled above and below basin highways. There is not sufficient information to draw conclusions similar to those of Goldman and Hoffman regarding direct Cl^- inputs from basin highways. But the general notion that the magnitude of road salt influence on those tributaries with limited road contact is small, appears to be valid. Thus, tributaries such as the Upper Truckee which crosses Highway 50 many times, Snow and Griff creeks which have Highway 267 traversing considerable portions of their watershed, and Third Creek which is adjacent to a portion of the Mt. Rose Highway, would be expected to be more heavily influenced by road salting operations. Such has been shown to be the case for at least the Upper Truckee River.

In addition to the tributary samples, several snow samples were collected. Fresh snow collected from Ward Valley had very low Cl^- content, consistent with values previously described. Roadside snow sampled at Cascade Creek had a slightly higher Cl^- content (1.214 mg/l). Since Cascade Creek lies within the "No-Salt Test Section," the increased salt content is probably "carry-over" from the salted sections of highway. Plow-packed snow from the business area of

Tahoe City had a very high Cl^- content of 250 mg/l. Snow melt, from packed snow draining into culverts, was noted to have a concentration of ~41 mg/l. Where such culverts release water directly into the lake, transient, localized increases in salinity would be expected.

Analysis of all lake samples obtained from the littoral (near shore) zone near Ward Creek indicated a mean Cl^- concentration of 2.054 mg/l (December 1980 to July 1981) and individual samples varied little from the mean (standard deviation = 0.166 mg/l). Although littoral chloride levels did decline by about 0.3 mg/l (from Appendix E, the difference between values obtained prior to and after mid-April) following the spring runoff, this decrease was certainly smaller than the >1 mg/l range reported previously for littoral locations (Joint Studies Reports, California Department of Water Resources 1972-74). Whether littoral Cl^- levels were elevated during the winter due to road salt inputs or whether the observed decline is simply a dilution effect due to the spring runoff would be difficult to determine. Mean Lake Tahoe chloride levels reported in the literature range from 1.9 mg/l (Brown, 1979) to 2.6 mg/l (T.R.P.A., 1971).

BIOASSAYS

In order to determine potential impacts of deicing agents on Lake Tahoe microbial communities (phytoplankton, bacteria), we used an extremely sensitive ^{14}C bioassay technique (Goldman, 1969). This was necessary because more conventional microbial growth assays (e.g. light-dark oxygen technique, in vivo fluorescence, direct biomass determinations, etc.) are not sufficiently sensitive to determine the extremely low rates of growth that occur in Lake Tahoe. Therefore, our basic experimental design involved the use of ^{14}C-labeled bicarbonate for estimating algal growth (autotrophic) and ^{14}C-labeled acetate

for estimating bacterial (heterotrophic) growth. The uptake of each was monitored as a function of the concentration of deicing agent (see methods for details).

A. Effects of Deicing Agents on Lake Tahoe Phytoplankton (Algal Bioassays)

1. Kiln-Dried NaCl

Lake Tahoe phytoplankton were neither significantly stimulated nor inhibited by 1 or 10 mg/l Cl^- additions of kiln-dried salt.* Bioassays using both levels of salt were conducted in December 1980, February, March, May, and June 1981. The benign effect of this salt at low concentrations appears to be a consequence of its low nutrient/contaminant content. As can be seen from Table 2, the macronutrients NH_4, NO_3 and Total-P, and the micronutrient Fe are all present in very low concentrations. Calculating the amount of nutrients contributed by a 10 mg/l Cl^- addition to lake water, only 0.04 µg/l NH_4, 0.02 µg/l NO_3, 0.03 µg/l Fe, and <.01 µg/l total-P increases above typical lake concentrations would be seen (Table 3). Such increases constitute less than 1% of the average ambient lake concentrations. Therefore, it seems highly unlikely that the phytoplankton would be significantly affected by such small increases and, in fact, our data support this conclusion. These findings are also consistent with those of the earlier study (Goldman and Hoffman, 1975) which found that low levels of kiln-dried salt did not affect the growth of Donner Lake phytoplankton. The desirability for continued use of this salt within the basin is thus reaffirmed.

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*Although significant growth responses to 1 and 10 mg/l as well as 0.01 mg/l Cl^- were indicated by the December 1981 assay, these results appear anomalous. While maximal algal growth responses compared to the control are typically observed on the 4th or 6th day of incubation, those for 0.1 and 10 mg/l but not 1.0 mg/l occurred on Day 2.

Table 2

RESULTS OF SELECTED DEICING AGENT MACRONUTRIENT AND MICRONUTRIENT CONTAMINANTS: REPORTED AS MG NUTRIENT/GRAM OF DEICING AGENT. N.D. = NOT DETECTABLE

	NH_4	NO_3	Fe	Total P	Na^+	K^+	Mg^{2+}	Ca^{2+}
Kiln-Dried NaCl	.0024	.0012	.0018	N.D.	-	-	-	-
Processed NaCl	.0036	.0006	.0158	N.D.	-	-	-	-
Sand Leachate	.0022	.0003	.0042	.0003	.0140	.0320	.0120	.0350
Cinder Leachate	.0012	.0004	.0012	.0028	.0910	.0530	.0130	.0330

Table 3

CONCENTRATIONS OF NUTRIENT CONTAMINANTS CONTRIBUTED PER BIOASSAY TREATMENT CONTRASTED WITH TYPICAL LAKE TAHOE CONCENTRATIONS. ALL CONCENTRATIONS REPORTED AS MICROGRAMS NUTRIENT/LITER. N.D. = NOT DETECTABLE

Treatment	NH_4	NO_3	Fe	Total P	Na^+	K^+	Mg^{2+}	Ca^{2+}
Kiln Dried NaCl								
.01 mg/l Cl^-	.00004	.00002	.00003	N.D.	-	-	-	-
0.1 mg/l Cl^-	.0004	.0002	.0003	N.D.	-	-	-	-
1 mg/l Cl^-	.004	.002	.003	N.D.	-	-	-	-
10 mg/l Cl^-	.04	.02	.03	N.D.	-	-	-	-
50 mg/l Cl^-	.2	.1	.15	N.D.	-	-	-	-
100 mg/l Cl^-	.4	.2	.3	N.D.	-	-	-	-
1000 mg/l Cl^-	4.0	2.0	2.0	N.D.	-	-	-	-
Processed NaCl								
1 mg/l Cl^-	.0006	.001	.026	N.D.	-	-	-	-
10 mg/l Cl^-	.06	.01	.26	N.D.	-	-	-	-
50 mg/l Cl^-	.3	.05	1.3	N.D.	-	-	-	-
100 mg/l Cl^-	.6	.1	2.5	N.D.	-	-	-	-
1000 mg/l Cl^-	6.0	1.0	26.0	N.D.	-	-	-	-
Sand Leachate								
0.1% of 10 g/l	.022	.003	.042	.003	.140	.320	.120	.350
1.0% of 10 g/l	.22	.03	.42	.03	1.40	3.20	1.20	3.50
Cinder Leachate								
0.1% of 10 g/l	.012	.004	.012	.028	.91	.530	.130	.330
1.0% of 10 g/l	.12	.04	.12	.28	9.1	5.30	1.30	3.30
Lake Tahoe Conc.	N.D.	2-20	2-30	<5-10	5.1	1.2	2.7	8.0

In assays using higher levels of kiln-dried salt, algal growth was inhibited to varying degrees. Concentrations as low as 100 mg/l Cl^- were observed to effect a sustained decrease in April and May whereas a concentration as high as 1000 mg/l only temporarily inhibited algal growth in March. The shifting critical salinity levels which caused sustained decreases in algal growth seemed to depend on the particular species assemblage and possibly on its nutritional state. Phytoplankton enumerations performed on both March and late May bioassays indicated the two species assemblages to be quite different. The March final control culture presented a diverse assemblage of species dominated by a "bloom" of the diatom Asterionella formosa, with Kephyrion c.f. rubri-claustri, a small microflagellate and an unidentified microalga all present in significant numbers; the late-May final control culture had lower cell densities overall, and a less diverse community. A microflagellate (<3 μ) was the dominant species, with Pseudokephyrion ovum, Chrysolykos planctonicus and an unidentified Chrysophyte also being quite abundant. It should be noted that the final cell numbers of all four of the predominant May species were reduced relative to the control after exposure to 100 mg/l Cl^{-1} kiln-dried salt, whereas in March only one of the four predominant species was similarly affected. Microscopic observations also suggested that the March culture was in a nutritionally favorable state, whereas the May culture may have been nutritionally deficient. Monoraphidium contortum, an alga typically found only in deep water (>75 m) (Vincent and Goldman, 1980) was abundant in surface waters on the date the March sample was taken. Vincent and Goldman indicated that such occurrences would only take place during winter mixing of epilimnetic water with the deeper nutrient-rich water in which these algae are found. The "bloom" of Asterionella formosa and other species in March seems to confirm such an influx of nutrients. Conversely, the May sample exhibited typical signs of nutrient deficiency in that

overall densities were low and many of the diatoms were observed to be chlorotic or senescing.

For purposes of this report, it suffices to say that levels of salt above 50 mg/l Cl^- kiln-dried salt are likely to inhibit algal growth at least at certain times of the year. Certain periods of increased tolerance to elevated salinities appear to correlate with periods in which the predominant species consist of those inherently more tolerant, and to periods of increased nutrient abundance.

2. Processed NaCl

Algal growth was significantly stimulated by a 10 mg/l Cl^- addition of processed NaCl on one of four dates assayed and was not significantly affected by 1 mg/l Cl^- additions in any experiment. Both levels of salt were assayed in March, May, June and early July 1981. Although not statistically significant, 10 mg/l Cl^- tended to be stimulatory ($P < .10$), in March carbon uptake 124% that of the control (i.e. the fewer replicates per treatment [2] utilized in this bioassay were responsible for the relatively more stringent statistical interpretation). In May, the same level of salt was significantly stimulatory, and ^{14}C-uptake increased to 121% of the control. In both June and early July, no significant response was observed. In the early July bioassay, the effects of 10 mg/l Cl^- were assayed on 4 different lake samples from 3 different areas of the lake: North Lake Tahoe ~50 m off the mouth of Snow Creek, West Lake Tahoe ~10 m off the mouth of Ward Creek, a West Lake Tahoe Pier ~300 m south of Ward Creek, and South Lake Tahoe ~10 m off the mouth of Tallac Creek. Each sample exhibited a different level of productivity, probably reflecting the different levels of available nutrients at each site, yet none of these samples responded to additions of the processed salt.

Chemical analysis of this salt revealed very low macronutrient (N and P) concentrations, similar to those of kiln-dried salt, but an elevated concentration of biologically available iron. This might be expected since processed salt is a combination of rock salt, with iron-containing sodium ferrocyanide, $Fe(CN)_6$, added as an anti-caking agent. Sodium ferrocyanide is soluble in water and has been reported to photodecompose and release cyanide when placed in direct sunlight (Hanes et al., 1970). However, photodecomposition apparently does not affect the amount of biologically available iron. Chemical analyses performed both on sunlight exposed and shaded solutions of processed salt revealed them to have a biologically available iron concentration of ~15.8 µg Fe per gram of salt. Therefore, the nutrients contributed by a 10 mg/l Cl^- addition of processed salt can be calculated to be 0.6 µg/l NH_4-N, 0.01 µg/l NO_3-N, N.D. Total-P, and 0.26 µg/l Fe. Approximately 9 times the biologically available iron contained in kiln-dried salt is contributed by a similar portion of processed salt.

Iron has been shown to be a nutrient limiting to Lake Tahoe phytoplankton during certain periods of the year (Goldman, 1965, 1972, 1976, 1981; Elder, 1974). Additions of 4-5 µg/l Fe have stimulated algal ^{14}C-uptake to 140% of control while nitrogen at the same levels had little effect (Goldman, 1974). Thus, the iron contributed by the processed salt may have been responsible for the observed stimulation in May and March. If the phytoplankton were in a sufficiently Fe-deficient state on these dates, one would expect them to be sensitive to the 0.26 µg Fe l^{-1} addition corresponding to a 10 mg/l Cl^- addition of processed salt. Phytoplankton cell counts performed on the late May initial lake sample indicated many of the diatoms to be chlorotic. This is often an indication of Fe deficiency (Bidwell, 1974), which further indicates that the algae, at least in this bioassay, may have been stimulated by the low levels of iron contained in the salt.

It is difficult to predict when such periods of Fe limitation would occur, since the interactions between nutrients and phytoplankton in Lake Tahoe are complex. One might expect to see limitation during periods of low iron input (mid-winter) and not to observe it during periods of higher input (spring). This is not necessarily the case though, as other nutrients may be similarly abundant or deficient during the same period. For instance, peak inputs of iron and nitrogen to Tahoe are typically associated with the spring snowmelt. Studies have indicated that inputs of iron from year to year are more directly dependent on the magnitude of the runoff than are those of NO_3-N (Leonard et al., 1979). Therefore, in a year of less intensive runoff, iron levels may regulate phytoplankton to a greater extent than nitrate levels. This may, in fact, explain the iron stimulation observed in May, since the spring runoff was lower in 1981 due to below average precipitation and snow-pack. The fact that nutrient-phytoplankton interrelationships are indeed complex and extremely dynamic is underscored by the lack of significant responses in June and July--as iron limitation in May might be expected to become more intense during these two months due to very low influx of iron. Though desirable for purposes of this study, it is difficult to predict the periods in which phytoplankton would be more or less likely to be stimulated by the iron contained in 10 mg/l Cl^- of processed salt.

As for kiln-dried salt, higher levels of processed salt were also assayed. A 50 mg/l Cl^- addition to Lake Tahoe water in late May proved to significantly inhibit algal ^{14}C-uptake by 21%. Uptake returned to control levels after four days and increased to 121% of the control on Day 6. Additions of 100 mg/l Cl^- significantly inhibited algal growth on two of three dates assayed. In April and late May, algal ^{14}C-uptake was reduced to 87% and 73% of the control, respectively, on Day 2. Unlike the response observed for kiln-dried salt (in

which algal ^{14}C-uptake remained below control levels), uptake returned to control levels after six days. In March, no initial inhibition was observed with this level of salt, and a significant increase to 140% of the control occurred after 8 days. This further emphasizes the fact that processed salt is more stimulatory than kiln-dried. A 100 mg/l Cl^- addition produced an inhibitory response similar to that produced by the same level of kiln-dried salt. Algal metabolism was initially reduced to 65% of the control and remained at approximately the same level throughout the bioassay.

Assays performed using higher levels of processed salt indicated an initial metabolic inhibition, identical to that caused by kiln-dried salt. However, phytoplankton inhibited by processed salt tended to recover to initial rates of ^{14}C-uptake better than those inhibited by kiln dried salt. It appears that the increased nutrients associated with this salt, particularly Fe, may have been responsible for this recovery. It should be noted that the 2.6 µg/l Fe contributed by a 100 mg/l Cl^- enrichment is approximately equal to the lower limit of the range of ambient lake concentration. Therefore, the salt addition was also causing an approximate doubling of the concentration of biologically available iron in the water.

3. Sand and Cinder Leachate

It is difficult to determine what level of abrasive per liter of water would be representative of levels observed in nature. A solution of 10 g abrasive per liter of distilled water was chosen for use in these bioassays only as a reference against which actually observed concentrations must be compared. Although no sediment analyses were performed on roadside snow, a previous study (Foster, 1971) determined the sediment load to be 1.53 g/l on the average, with a maximum of 4.0 g/l in snow disposal site snow, which can be considered a rough

approximation of roadside abrasive levels. However, this sediment consists not only of abrasive, but also includes silt and other particulates, and so the leachate solutions prepared for use in the bioassays represent somewhat high concentrations. However, it should be remembered that a variety of conditions are present in the field which may further enhance leaching action. For instance, the chemical composition of the snowmelt water itself, whether draining off the pavement or percolating through disposal mounds, is likely to be very different from the distilled water used in preparation of the leachate solutions. Obvious differing factors are solute content and pH. Lake Tahoe precipitation is slightly acidic and an acidic leaching solution would be expected to more efficiently leach chemically bound substances than would a more neutral one. Abrasives on the highway are also subjected to grinding and mechanical breakdown. Such wear would be expected to increase the surface area of particulates, which would further tend to increase leachate levels. Thus, the solutions prepared may actually provide a fairly good approximation of the leachate concentrations found in the field.

Sand- and cinder-leachate solutions were assayed in April and June 1981. A 5 ml cinder leachate addition (1% by volume) was significantly stimulatory in April (127% of control), while a 0.5 ml addition (0.1% by volume) was only slightly stimulatory (110% of control) and not significantly different from the control. Sand leachate proved to be neither significantly stimulatory nor inhibitory during this same period. Neither sand nor cinder leachate was significantly stimulatory in June. Although the 0.1% sand leachate showed statistically significant stimulation on Day 2 (in June), this result was anomalous, since a 1% concentration showed no effect.

Chemical analysis of the macro and selected micronutrients leached from both abrasives, as well as the nutrient levels contributed by each enrichment

are given in Tables 2 and 3. It can be seen that both abrasives contribute very low concentrations of the nutrients analyzed. Sand contributed slightly more NH_4 and 3-4 times more iron than cinder, whereas cinder contributed considerably more total-P and sodium. The stimulatory effects of these low levels of nutrients will again depend on the nutrient-phytoplankton interrelationships at the time of sampling. The much higher level of total-P in the cinder leachate may have been responsible for the stimulation seen in April. It is also possible that a particular combination of total-P with other limiting nutrients, possibly trace elements, contained in the cinder leachate were jointly responsible. The results of the cation analysis indicated that cinder leachate contained a greater quantity of the monovalent cations sodium and potassium than did sand leachate. Levels of the divalent cations Mg^{+2} and Ca^{+2} were similar in both solutions. It is probable that Na^{+} and K^{+} as well as the total-P are actually solubilized from the cinder material itself and that sand, which generally consists of minerals which are more resistant to weathering, releases lower quantities of these materials.

The fact that cinder was stimulatory during certain periods of the year may be important to decisions regarding its usage. In addition to the leachate nutrients carried in runoff, cinder may be contributed directly as a particulate to the lake. It has been suggested that constant vehicular traffic over roads on which cinder has been applied has a tendency to break down the cinder particles and resuspend them into the air as a fine dust. Cahill et al. (1978) previously showed that the levels of particulates (as traced by silicon) in the air near major thoroughfares, during the winter, can actually approach concentrations observed near dirt roads in the summer. He attributed these high winter levels of particulates to road sanding and salting operations. Since most basin highways are located adjacent to the lake, much of this suspended

particulate material is likely to eventually settle in the lake and so become a direct source of nutrients and silt. Sand appears to be the more biologically inert of the two abrasives, and so its use may be more desirable than cinder within the Tahoe basin.

4. Snow, Stream Runoff, Roadside Drainage

In addition to testing the effects of individual deicing agents, several snow, roadside drainage and stream runoff samples were assayed in an attempt to approximate the impacts of deicing-agent-influenced and noninfluenced waters reaching the lake. Although natural waters contain such a broad spectrum of contaminants that it is difficult to attribute the results observed to any one substance, these assays are valuable for comparing the stimulatory or inhibitory potential of different waters.

Cascade and Eagle Creek waters, which traverse the "No Salt" test section of Highway 89, both inhibited algal growth. Eagle Creek water appeared almost toxic in its effects on the phytoplankton as algal growth was reduced by 85-90%. Since the highways impacting these streams are not subject to salting, deicing salts did not play a role in this inhibition. Eagle Creek traverses a heavily used summer day-use area; it is possible that some contaminant was derived from this area. General Creek marsh water and Ward Valley snow, which also had natural levels of chloride, proved to be neither stimulatory nor inhibitory to algal growth. An assay performed in which kiln-dried salt in concentrations of 1 and 10 mg/l Cl^- was added in concert with the Ward Valley snow produced similar results. Although basin snow may contain significant quantities of NO_3, NH_4 and SO_4, it probably represents the "purest" water reaching the lake. The results of the snow + kiln-dried salt experiment suggest that this salt, at least at concentrations of about 10 mg/l, is equally innocuous in both natural and

laboratory "pure" waters. Ward Creek water sampled from above and below Highway 89 significantly stimulated algal growth to 127% and 118% of the control, respectively. Although chloride levels in this tributary were slightly elevated compared to the west shore average, the highway itself did not add to the Cl^- concentration of Ward Creek. Therefore, the stimulatory potential of these waters appears to result from natural contamination added prior to reaching the highway.

Samples which showed minor road salt contamination were observed to have either no effect or a stimulatory effect on algal growth. A roadside drainage sample with a chloride content of 8.555 mg/l Cl^- had no significant effect on algal growth, whereas a Cascade Creek roadside snow sample, which showed obvious cinder contamination but little road salt contamination (1.214 mg/l), significantly stimulated algal growth to 164% of the control after eight days. The high cinder contamination may have been partially responsible for the stimulation, but it should be recognized that several other road derived contaminants may have also been present.

Water samples having a high degree of road salt contamination also significantly stimulated algal growth. But because the contaminating salt in these samples was the non-stimulatory kiln-dried salt, stimulation was probably attributable to the other nutrient contaminants. A Tahoe City roadside snow sample (250 mg/l Cl^-) caused the highest stimulation (173% of the control after eight days) observed in these assays. Contamination with road-derived substances other than salt was visually noticeable in this sample. A sample of water leaching from roadside sand on the Ward Creek bridge stimulated algal growth to 138% of the control after eight days. The stimulatory potential of these waters is probably typical of drainage from heavily traveled sections of basin highways and urban areas. In particular, where contaminated water is channeled

directly to the lake via storm drainage systems, little dilution with "clean" snowmelt would occur, and at least on a very localized scale, impacts would be greatly increased.

B. Effects of Deicing Agents on Lake Tahoe Heterotrophs (Bacterial Bioassays)

1. Kiln-Dried NaCl

Lake Tahoe bacterial metabolism, as measured by ^{14}C-acetate uptake, was neither significantly stimulated nor inhibited by kiln-dried salt concentrations of 100 mg/l Cl^- or lower. Bioassays were conducted twice in May using 1, 10, and 100 mg/l Cl^- concentrations and once in June for 1, 10, and 50 mg/l chloride levels. A much higher level of this salt, 1000 mg/l Cl^-, was assayed in early May and found to greatly increase bacterial metabolism to 236% of the control.*

As in the algal growth experiments (see Section V.A.1) kiln-dried salt had no effect on bacterial metabolism at the low concentrations measured in tributaries entering the lake. Again this appears to be due to its low nutrient/contaminant content. Although less research has been done on Lake Tahoe heterotrophs relative to autotrophs (algae) with regard to nutrient limitation, iron, nitrogen, and phosphorus are all expected to be in sufficiently short supply to have a limiting effect on bacterial growth, as well as algal growth. More important though is the availability of dissolved organic carbon

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*NOTE: In all bacterial bioassays, a high degree of variability was noted between replicates for particular treatments. Because of the tendency of bacteria often to occur as aggregates, as well as grow in association with detrital particulates (Paerl, 1974), equal distribution of bacterial populations in culture flasks is not always achieved. Furthermore, the bacteria may be in varying physiological states in different culture flasks. For instance, some of the bacteria associated with stream-derived detritus may be in a poor state, due to their inability to do well in a lake environment. McKinley and Wetzel (1979) noted similar high variability in culture studies of Lawrence Lake heterotrophs, as did Goldman and Hoffman (1975) in the previous study. Thus, the high degree of variability in bioassays using indigenous lake bacterial populations is not unusual.

(DOC) which is present in very low concentrations in Lake Tahoe. The DOC consists of solubilized material from both living and dead organisms and is the principal source of energy for heterotrophic metabolism. Kiln-dried salt has already been shown to contribute only very low concentrations of NH_4, NO_3, Total-P and Fe (Section V.A.1) and it would not be expected to contain significant amounts of organic carbon.

The bacteria were not affected by concentrations of kiln-dried salt which were sufficient to reduce algal growth. Bacterial metabolism was affected only at very high concentrations and in these cases the observed response was exactly opposite (stimulatory) to that of the phytoplankton. It is possible that the nutrients added along with the salt at these high levels were responsible for the observed growth. It is also possible the salt had an indirect rather than a direct effect on the bacteria. The positive response may have been a consequence of the detrimental action of the salt on the algae. Some algae respond to osmotic stress by releasing organic solutes (Antonyan and Pinevich, 1967), whereas other species are not able to react sufficiently and experience cell lysis. In both situations there is a net release of DOC which may actually have provided the bacteria with organic material sufficient to stimulate growth above the control.

2. Processed NaCl

Bioassays using 1 and 10 mg/l Cl^- concentrations were conducted twice in May and once each in June and July. Bacterial metabolism was significantly inhibited by a 10 mg/l Cl^- addition of this salt on only one of the four assay dates. In June, acetate uptake was reduced to 83% of control after two days by 10 mg/l Cl^- with a subsequent decline to 45% of the control by Day 6. The 1 mg/l Cl^- treatment also tended to be inhibitory ($P < .10$) at this time with metabolism reduced to 60% of the control by Day 6. Higher levels of salt were

assayed in May, and a 1000 mg/l Cl^- enrichment was required to cause inhibition similar to that observed for only 10 mg/l in June.

At least at certain times of the year, the bacteria appear to be detrimentally affected by some chemical or combination of chemicals derived from this salt. The varying toxicity of this salt is not easily explained though. It appears that either the toxicity of the chemical(s) or the susceptibility of the bacterial populations must be subject to dynamic change. Cyanide derived from the ferrocyanide of processed salt is both a highly toxic compound and one whose toxicity may vary depending on the environmental conditions. As mentioned earlier, ferrocyanide rapidly degrades in sunlight but slowly degrades in the absence of light to release cyanide anions (Hanes et al., 1970). The toxicity, though, is not dependent solely on the action of the CN^- anion but on its combination with the hydrogen ion H^+ to form HCN (Brown, 1968). Therefore, at lower pH, when there is a higher concentration of H^+ available to complex with CN^-, the toxicity of cyanide is enhanced. For instance, at pH 7 or below, 99 percent of the cyanide exists as HCN, whereas at pH 9 only approximately 58 percent exists in the toxic form (Hanes et al., 1970). The waters of Lake Tahoe and its associated tributaries have been shown to be subject to localized pH fluctuation with values occasionally observed as low as 6.8 and as high as 8.4 (California Department of Water Resources, 1972, 1973, 1974). A lower pH at the sampled littoral site in June relative to other dates could acount for an increased toxicity of the cyanide fraction of the processed salt and, therefore, the bacterial inhibition observed. Unfortunately, pH measurements were not included as a parameter of study in this research.

The levels of cyanide which become inhibiting or toxic to bacteria are reported to vary depending on the species. Gel'man et al. (1967) noted concentrations of between 10^{-3} and 10^{-4}M CN^- to cause nearly total inhibition

in several species of bacteria. Zintgraff (1968) noted unacclimated mixed bacterial populations to be affected most severely, with concentrations as low as 1×10^{-7}M observed to affect the growth response. The concentrations of CN^- applied with a 10 mg/l Cl^- addition of processed salt in this study appear to be very low. Using the biologically available iron as an estimate of the iron contributed by the ferrocyanide in processed salt, and assuming a ratio of 6 CN^-: 1 Fe and a concentration of 0.23 µg/l biologically available Fe, the cyanide contributed to solution would be ~2.5×10^{-8}M. This value is probably the minimum value contributed since not all the Fe is accounted for in a determination of biologically available iron. While this concentration is very low, it is close (within an order of magnitude) to values which have previously been observed to affect microbial populations. As indicated earlier, the particular action of the salt may also be related to the susceptibilities of the specific bacteria involved. Therefore, since bacterial populations in lakes are extremely dynamic both spatially and temporally (Wetzel, 1975), the somewhat inconsistent effects we observed between four trial dates in May and July are not especially surprising.

Whether the source of inhibition is cyanide, species-specific sensitivities or even some other contaminant, the observed potential for bacterial metabolic inhibition as well as its potential for algal growth stimulation at low concentration suggests that the use of processed salt may be less desirable than the use of kiln-dried salt. It is recommended, therefore, that Caltrans continue the exclusive use of kiln-dried salt in the Tahoe basin.

3. Sand and Cinder Leachate

The bacterial response to sand and cinder leachate solutions was determined in June 1981. Similar to its effects on algal metabolism, cinder leachate appears to stimulate bacterial metabolism. A 1% solution of cinder leachate significantly

stimulated bacterial metabolism to 126% of the control on Day 2. Metabolism subsequently returned to control levels by Day 6. A 0.1% solution had no apparent effect. Sand leachate proved to be neither significantly stimulatory nor inhibitory during this same period.

Phosphorus is an essential nutrient required by both the bacteria and the algae for growth. As indicated earlier, cinder leachate contributed proportionally more phosphate to solution than NH_4, NO_3 or the micronutrient iron. Goldman and Hoffman (1975) previously reported the deicing agent potassium phosphate to be highly stimulatory to bacterial growth, causing increases in ^{14}C-acetate uptake of up to 400% of control. Thus, the extremely low levels of phosphorus contributed by the 1% addition of cinder leachate may still have been sufficient to stimulate both bacterial and algal growth in the present study. Sand appears to be the favored abrasive for use within the Tahoe basin since, even at enrichments of 1%, it had no significant effects upon either algal or bacterial metabolism.

C. Periphyton Bioassay

In Lake Tahoe, the periphyton (attached algae) community has provided the most visible evidence of cultural eutrophication in the basin. Because the littoral zone is a transition region between the terrestrial environment and the pelagic environment of lakes, it is first to be exposed to increased nutrient inputs which often result from man's activities within the drainage basin (Goldman, 1981; Loeb, 1980). Therefore, we felt it was essential to conduct a preliminary experiment designed to test for the potential effects of deicing salts on the growth of Lake Tahoe periphyton.

Continuing studies of the periphyton at Lake Tahoe have shown that nitrogen-fixing blue-green algae comprise a major fraction of the periphyton

community below the uppermost "splash" zone (Loeb, 1980; Goldman, 1981; Loeb and Reuter, 1981). These algae have the ability to convert biologically unavailable atmospheric nitrogen gas (N_2) into ammonium which can then be directly used for cellular nitrogen metabolism. Not only do the blue-greens benefit from this process, but other non-fixing periphyton and planktonic algae may benefit from the eventual death and release of this new, external source of combined nitrogen. Furthermore, sodium, and particularly iron, have been shown to be essential to the enzymatic (via nitrogenase) reduction of N_2 to NH_4 (Stewart, 1974), and both of these nutrients have been previously shown to be contributed by either kiln-dried (Na) or processed (Na + Fe) salt.

Most bioassay studies of periphyton have used algae grown on artificial substrates, such as plastic or glass slides, which are usually suspended in the water for periods of a few weeks. However, since the blue-green algae-dominated communities at Lake Tahoe are very slow to recolonize a substrate (either artificial or natural), we used a new technique involving in situ incubations of epilithic periphyton with salt solutions and ^{14}C-bicarbonate administered using SCUBA techniques (Loeb, 1981). Algae were pre-incubated in hemispherical domes with 1 and 10 mg/l Cl^{-1} of kiln-dried and processed salt for 24 hours. Carbon-14 labeled-sodium bicarbonate was then injected into the chambers followed by a 3.3 hour incubation after which the algae were subsampled and analyzed for radioactivity, and carbon and nitrogen content.

The results indicated no statistically significant differences ($P > 0.05$) between treatment and control rates of uptake. However, values of specific-productivity (i.e., productivity normalized to biomass) were always less in treatments than in controls indicating a general depression of photosynthesis in the presence of increased salt concentration. Future studies of algal-salt

interactions should be expanded to include assays of periphyton growth using longer incubations and assays for N_2-fixation which may be more sensitive to road salt contaminants.

Figure 3. Experiments analyzing the effects of deicing salts on Lake Tahoe periphyton growth were performed in situ. Photo above shows a Tahoe Research Group diver in the littoral (nearshore) zone of Lake Tahoe with a similar periphyton bioassay in progress. (Photo by Flip Nicklin, National Geographic Society, 1980)

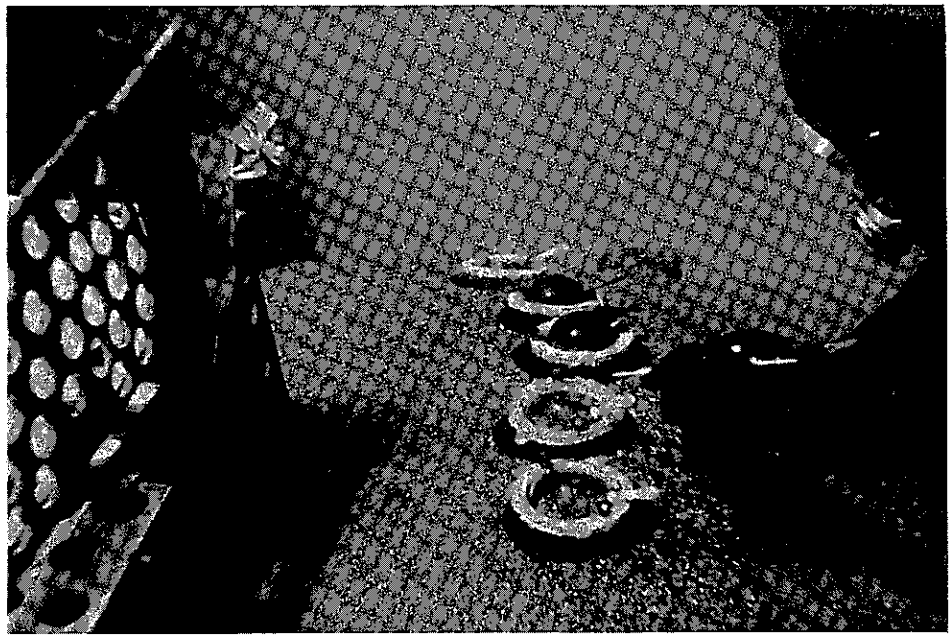

Figure 4. Unique incubation chambers are affixed by weight over substrate covered with attached algae (periphyton). Small quantities of solutions containing the deicing agents are then injected into the chambers (shown above) and periphyton allowed to incubate for 24 hours. 14C-bicarbonate is then added to each and rates of photosynthesis determined.

V. CONCLUDING DISCUSSION

Results of the stream sampling done in the Lake Tahoe basin both in 1974-75 and this past winter (1981) showed that the majority of these streams exhibited low chloride concentrations (near natural levels) throughout the winter. This may indicate that either brief pulses of increased chloride levels do occur in response to deicing operations but have gone undetected due to insufficiently intense sampling, or that road salting activities actually have little chloride-impact on basin streams. It does not indicate that applications of road salt are insignificant relative to the runoff contributed from the watershed. The 412 tons of road salt applied during winter 1980-81 to basin highways on the California side of the lake would have been sufficient to maintain chloride concentrations of 0.7 mg/l above ambient for a full year in each of the California-side tributaries.* Very few of the basin streams showed such elevated levels.

If, indeed, tributaries contribute very little of the salt applied to basin highways, the question remains as to where it is going. It may be that direct surface runoff or groundwater flow contribute the major influxes of road salt to the lake. The close proximity of highways to the lake in several areas suggests that direct inputs of saline street runoff are likely. In populated areas, surface runoff may be channeled directly into the lake via storm drainage systems. Groundwater inputs are also likely. The occurrence of salt-damaged trees along sections of basin highways (Scharpf and Srago, 1974; Leiser et al., 1980) indicates that at least a portion of the saline runoff enters soils adjacent

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*Assuming an average annual tributary input of 269,000 acre feet on the California side of the Basin (Dugan and McGauhey, 1974) and that 412 tons of road salt (60.66% Cl^-) reached the lake this same year via tributaries. Note that runoff 1980-81 was reduced compared to the average, thus 0.7 mg/l is probably an underestimate.

to salted sections of highway. In many areas, these soils overlie porous alluvial and glacial deposits through which groundwater may flow into the lake (U.S. Department of Agriculture, 1974; Loeb and Goldman, 1979). It is conceivable that much of the salt input into the surface soils is eventually flushed downward through the alluvium into the groundwater. Leiser et al. (1980) in fact found the soils in their study area to leach well, with no evidence of salt build up. Once in the groundwater the salt must eventually end up in the lake. Further study on the contribution of groundwater Na and Cl^- to Lake Tahoe is necessary to assess the magnitude of this salt source relative to other pathways into the lake.

With respect to surface runoff and the fate of the salt, a further point deserves mention. One of the proposals to deal with non-point source pollution within the basin is to channel storm runoff through marshland-wetland systems where natural sedimentation and removal of nutrients takes place. In such systems, saline street runoff may be retained for long periods of time and dissipate at slow rates. The consequences of increased salinities in such areas are uncertain. Little effect might be expected on the aquatic vegetation, as most of the macrophytes die back in late fall. But salt may move from the water into the marsh sediments (Lerman and Jones, 1973) and have significant detrimental impacts on microbial communities as well as on buried macrophyte roots and rhizomes. On the other hand, it may be that such systems will not be adversely affected by these salt concentrations, and may actually serve to mitigate the effects. It is recommended that preliminary studies be conducted prior to adopting a disposal scheme based on use of wetlands for water treatment.

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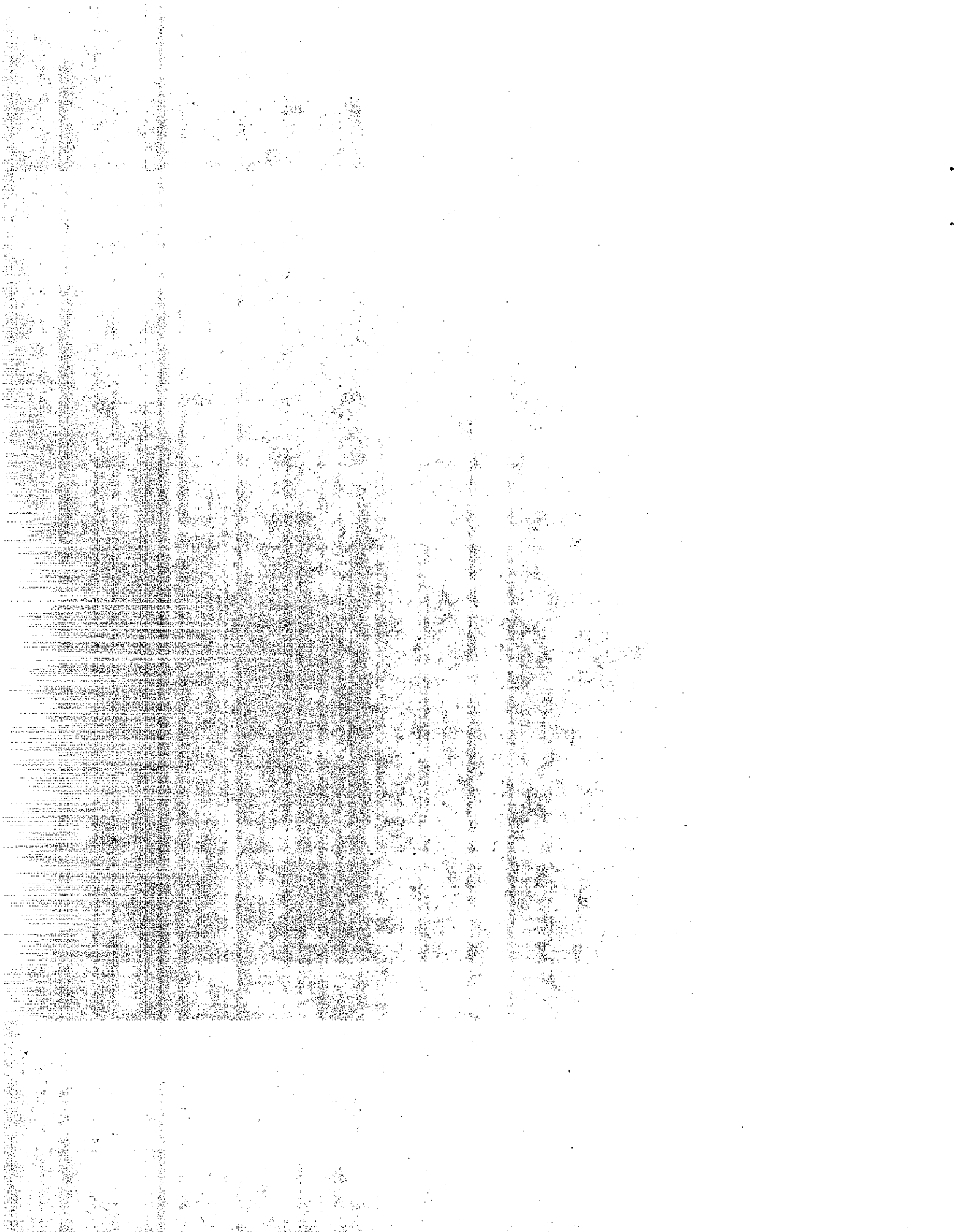
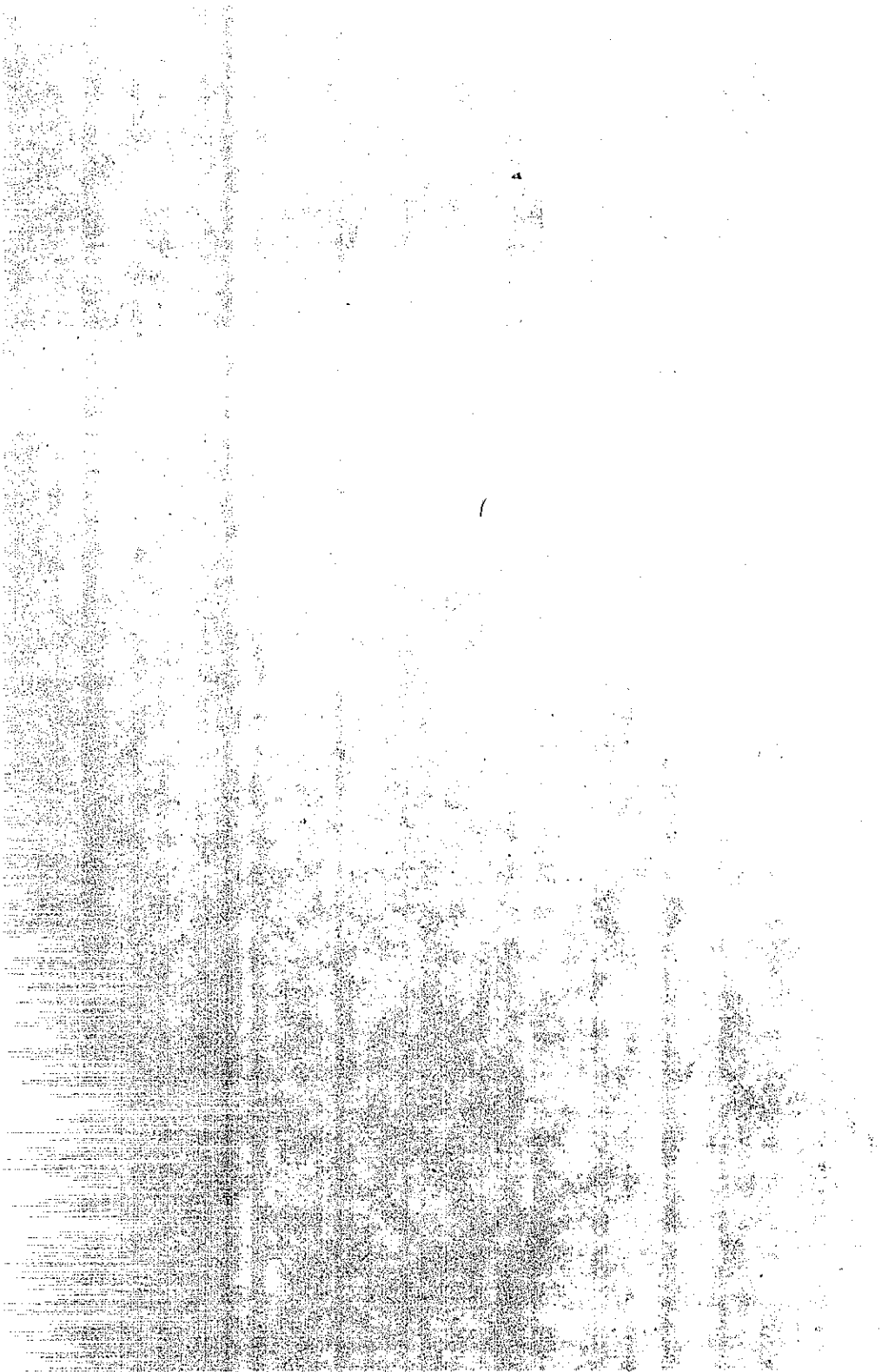


APPENDIX A

Graphical Presentation of Statistically Significant Bioassay Results

* Statistically significant.



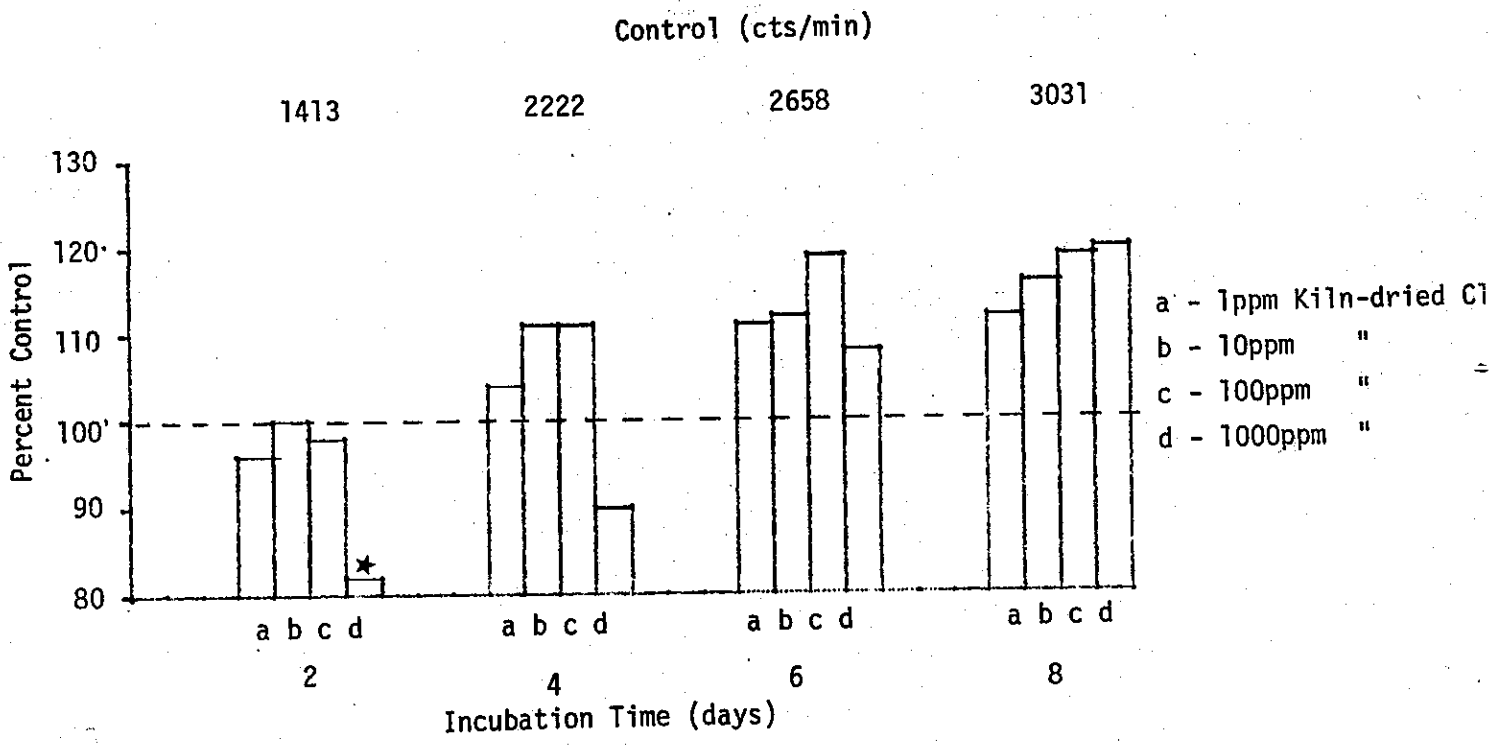


Figure 1. Bioassay of kiln-dried salt additions to L. Tahoe water conducted 11-19 March 1981. (Algal)

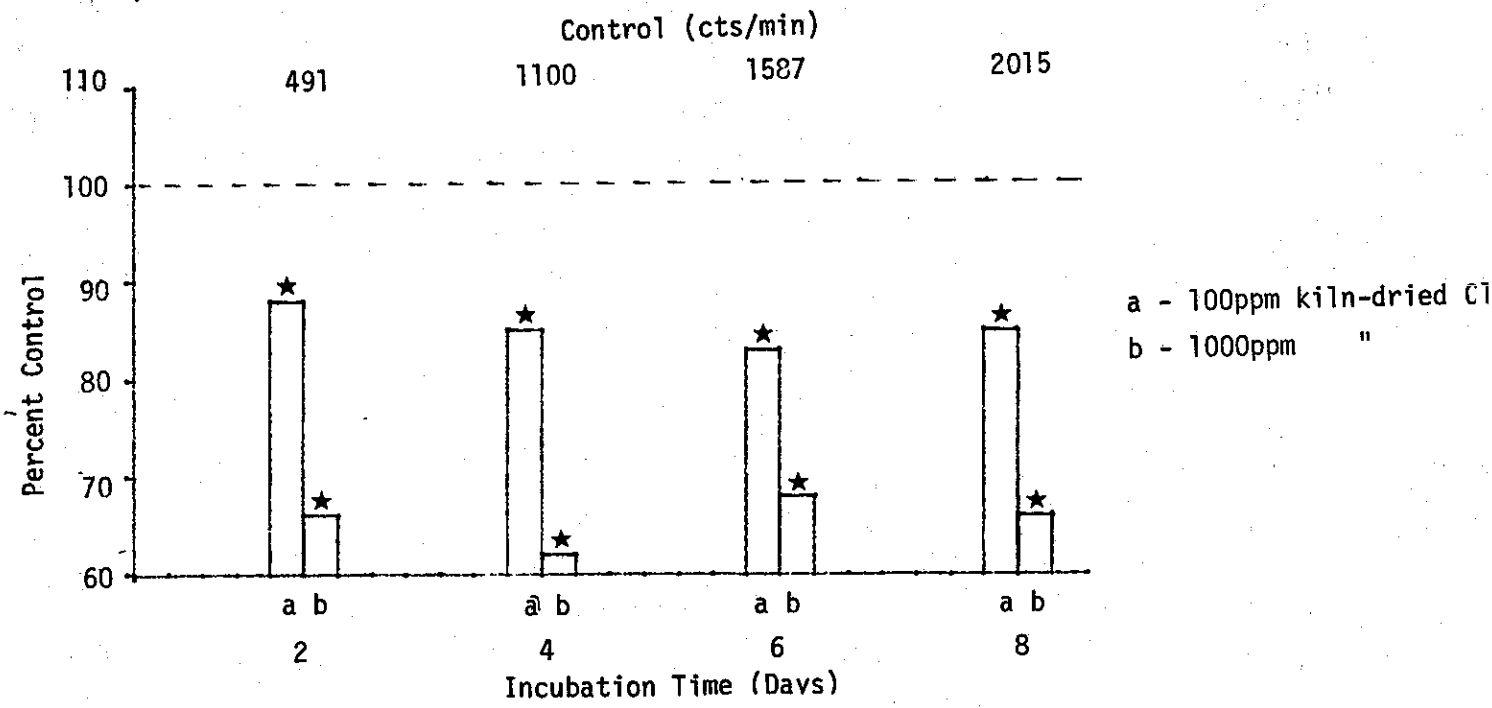


Figure 2. Bioassay of kiln-dried salt additions to L. Tahoe water conducted 3-11 April 1981. (Algal)

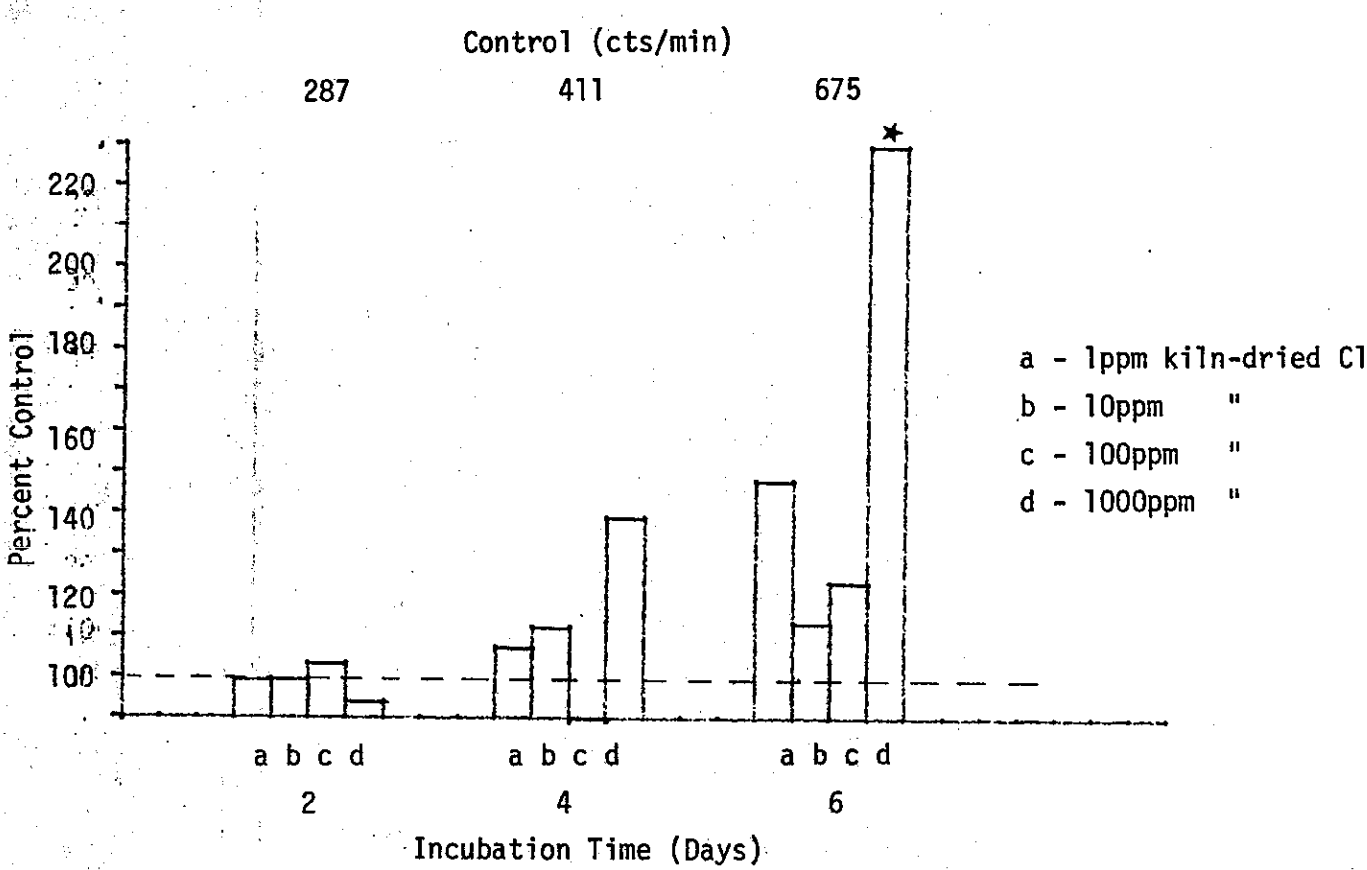


Figure 3. Bioassay of kiln-dried salt additions to L. Tahoe water conducted 4-10 May 1981. (Bacterial)

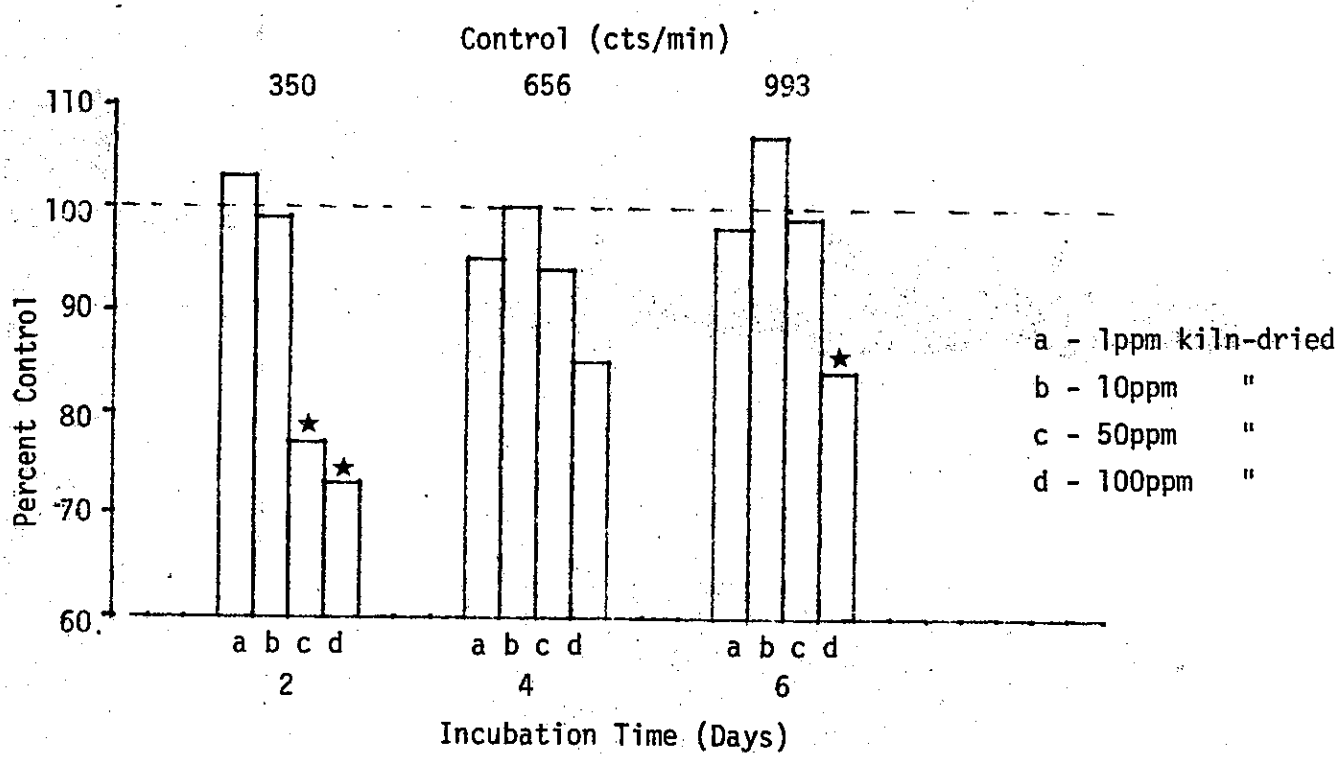


Figure 4. Bioassay of kiln-dried salt additions to L. Tahoe water conducted 30 May - 5 June 1981. (Algal)

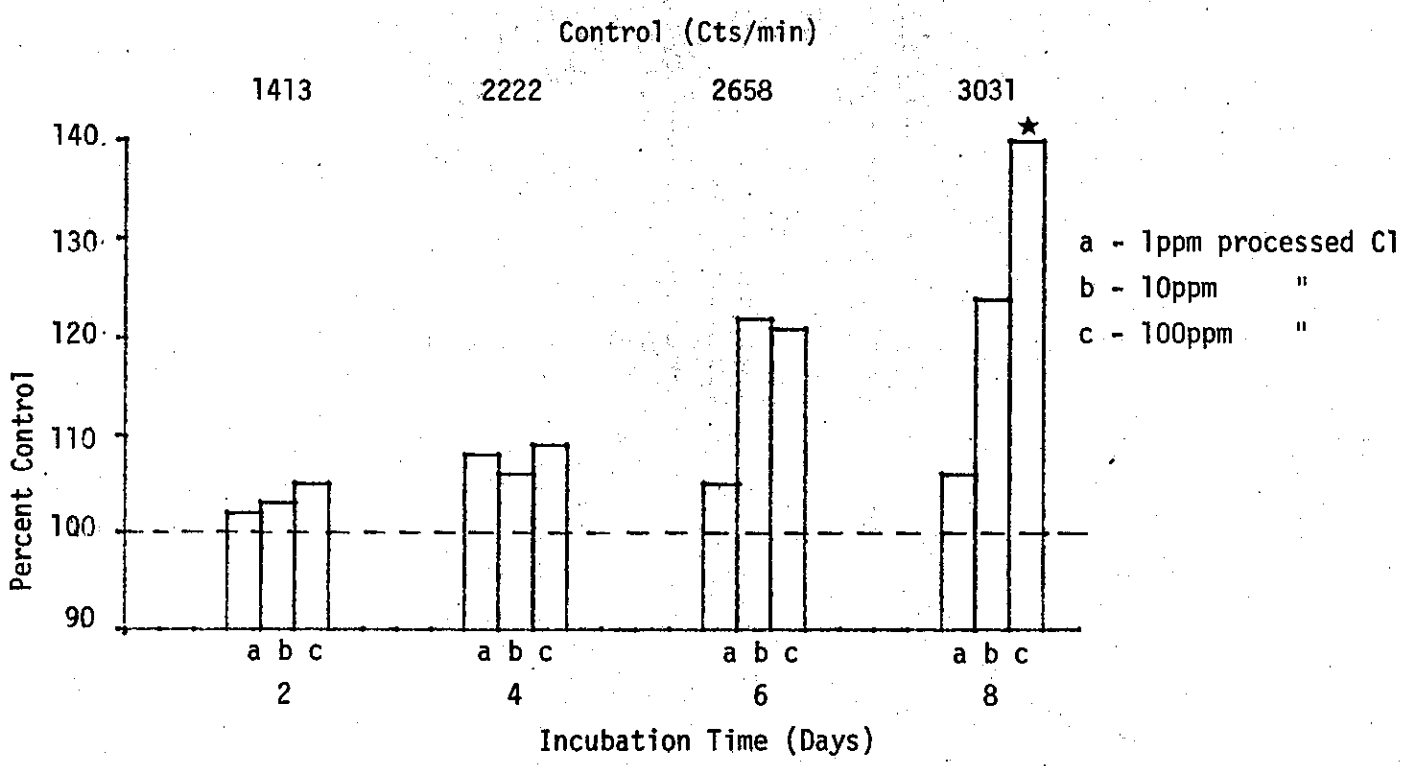


Figure 5. Bioassay of processed salt additions to L. Tahoe water conducted 11-19 March 1981. (Algal)

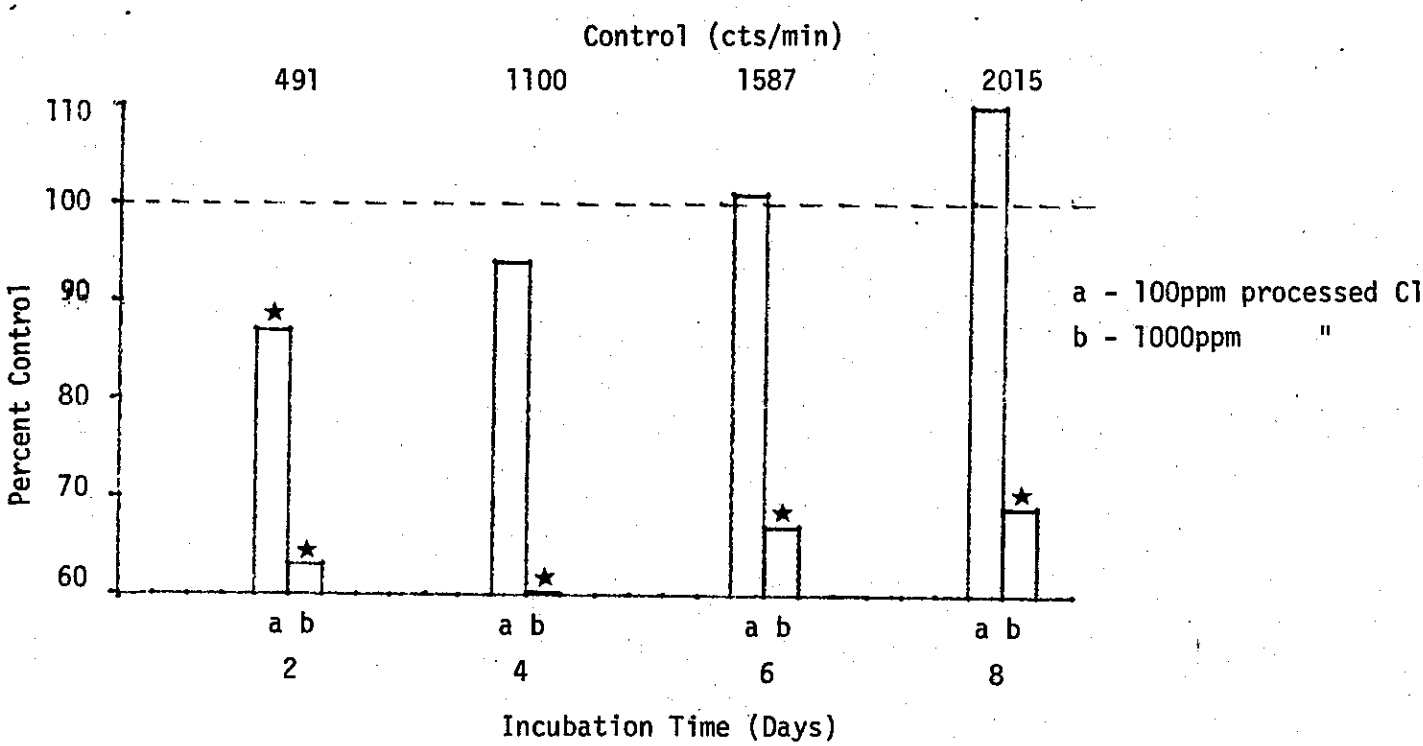


Figure 6. Bioassay of processed salt additions to L Tahoe water conducted 3-11 April 1981. (Algal)

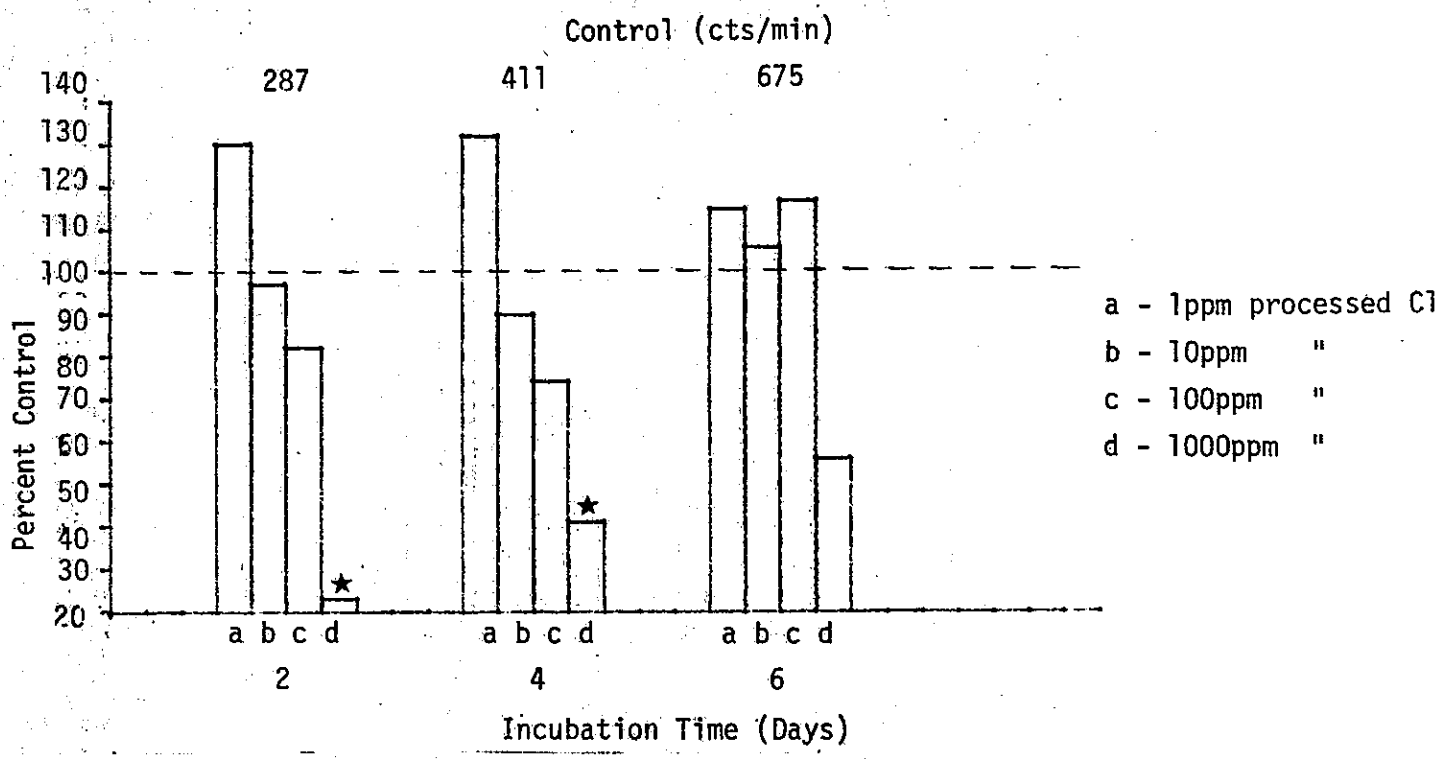


Figure 7. Bioassay of processed salt additions to L. Tahoe water conducted 4-10 May 1981. (Bacterial)

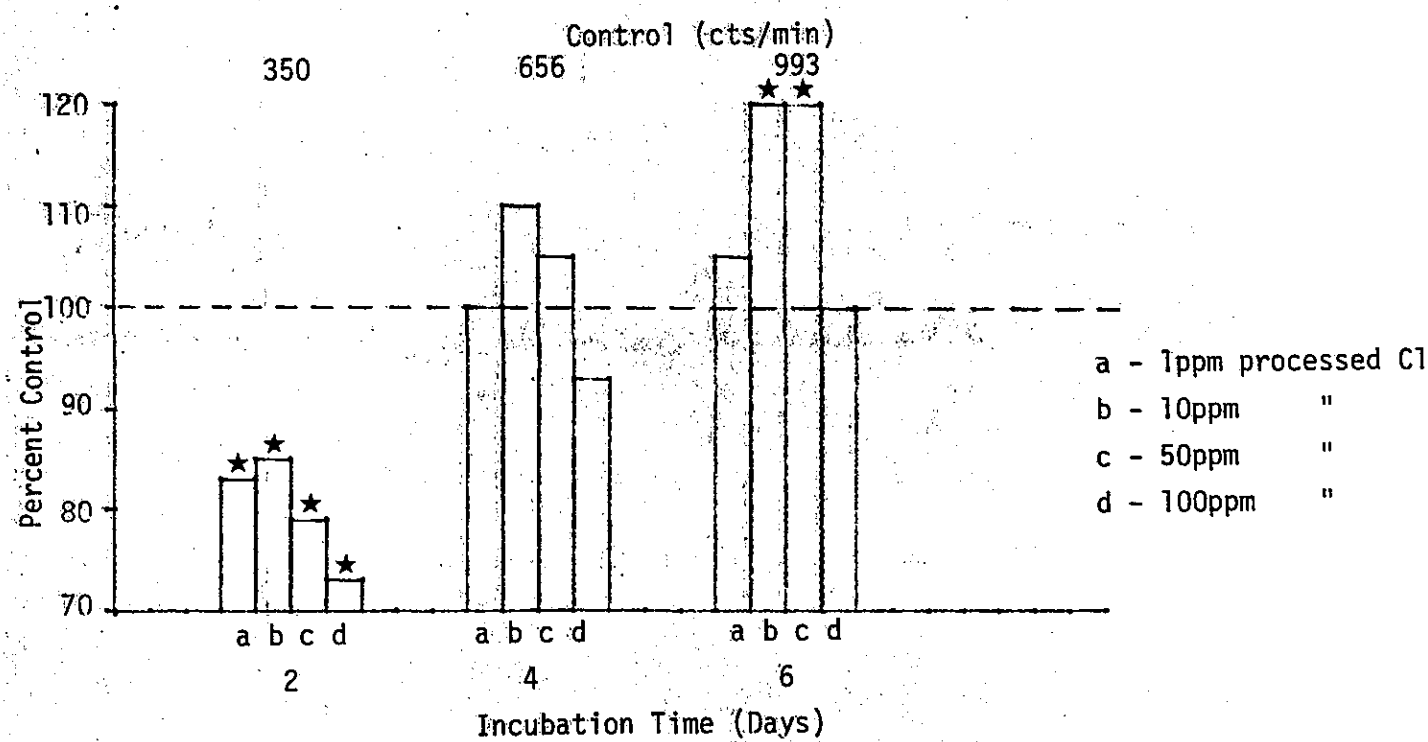


Figure 8. Bioassay of processed salt additions to L. Tahoe water conducted 30 May - 5 June 1981. (Algal)

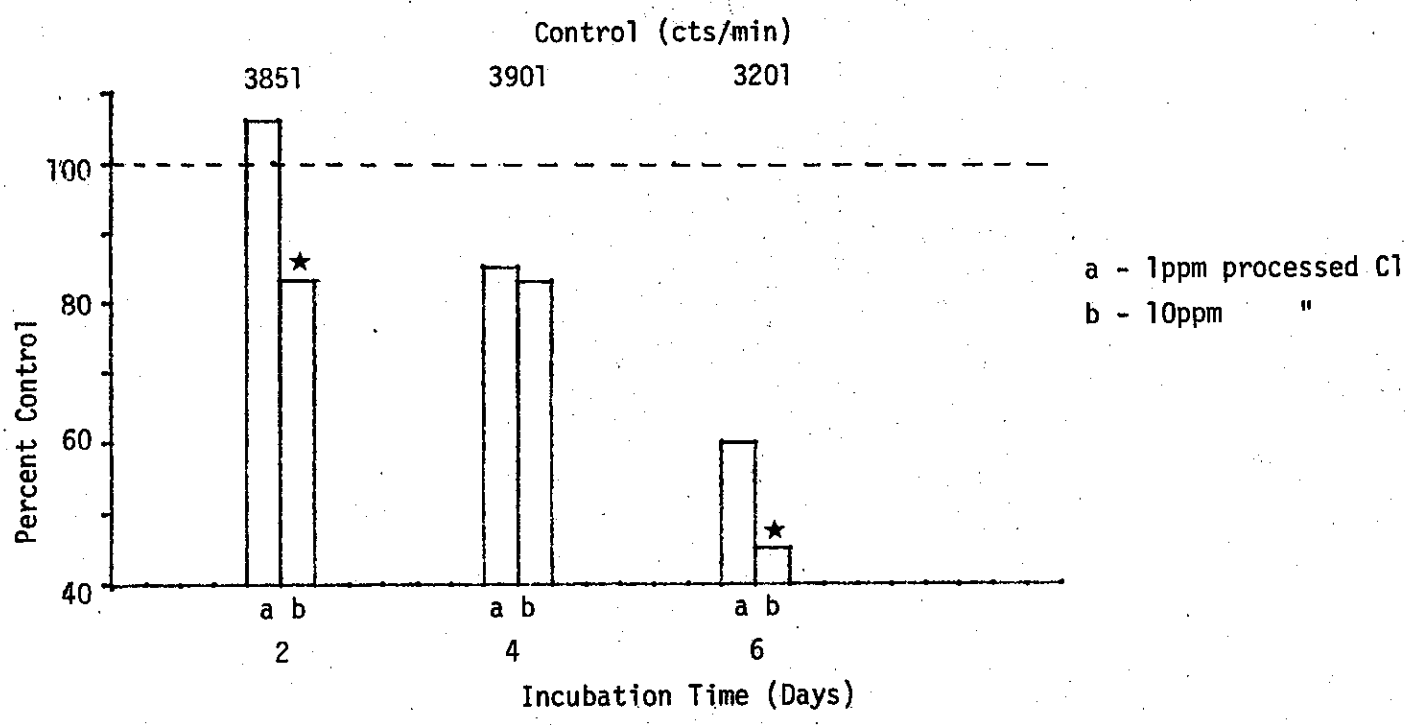


Figure 9. Bioassay of processed salt additions to L. Tahoe water conducted 23-29 June 1981. (Bacterial)

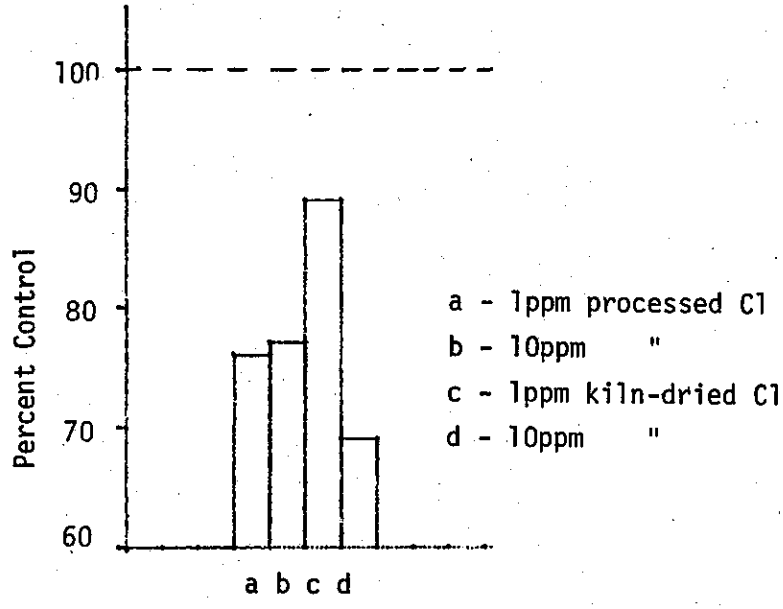


Figure 10. Periphyton bioassay conducted in situ at Rubicon Pt. 7-8 July 81.

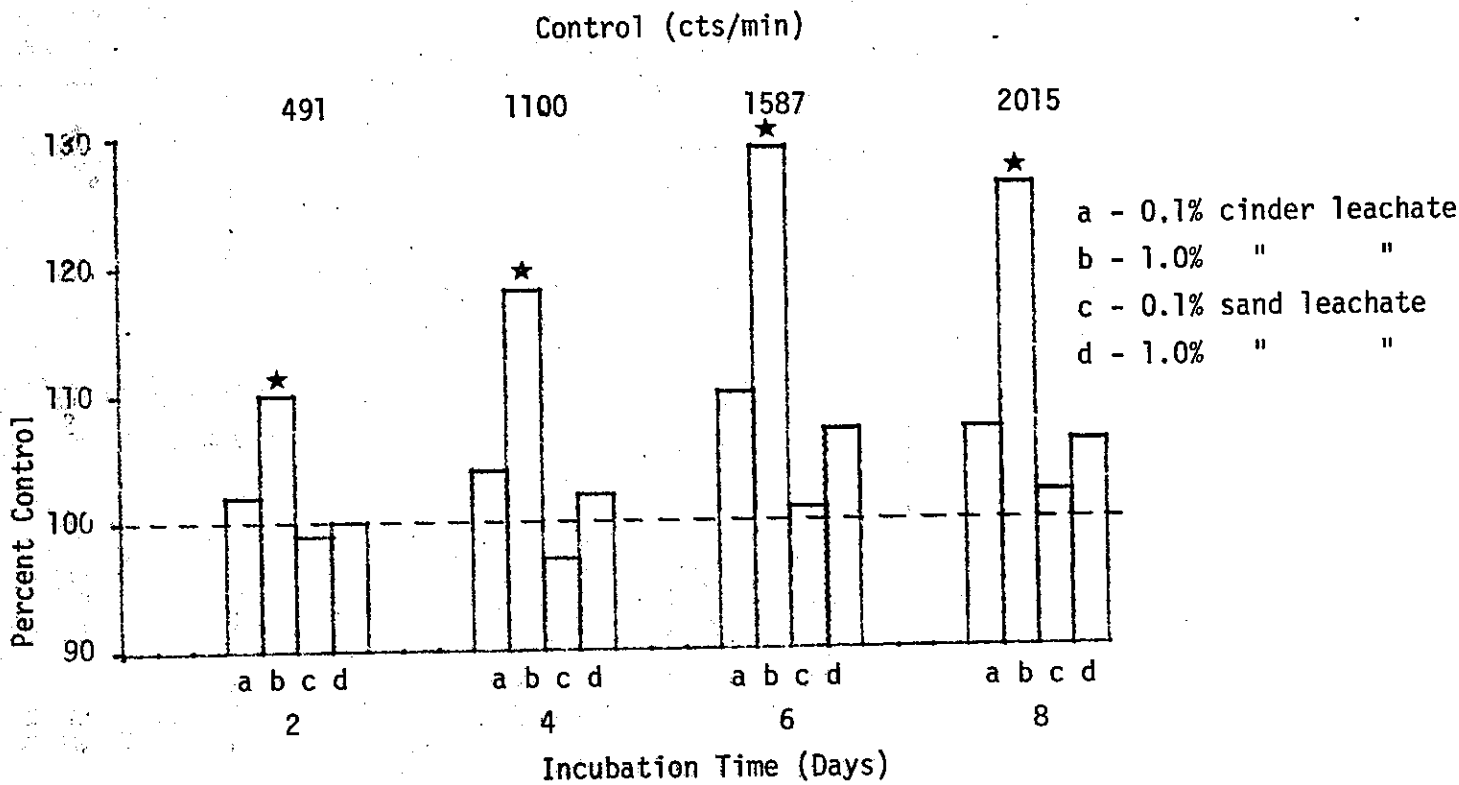


Figure 11. Bioassay of sand and cinder leachate additions to L. Tahoe water conducted 3-11 April 1981. (Algal)

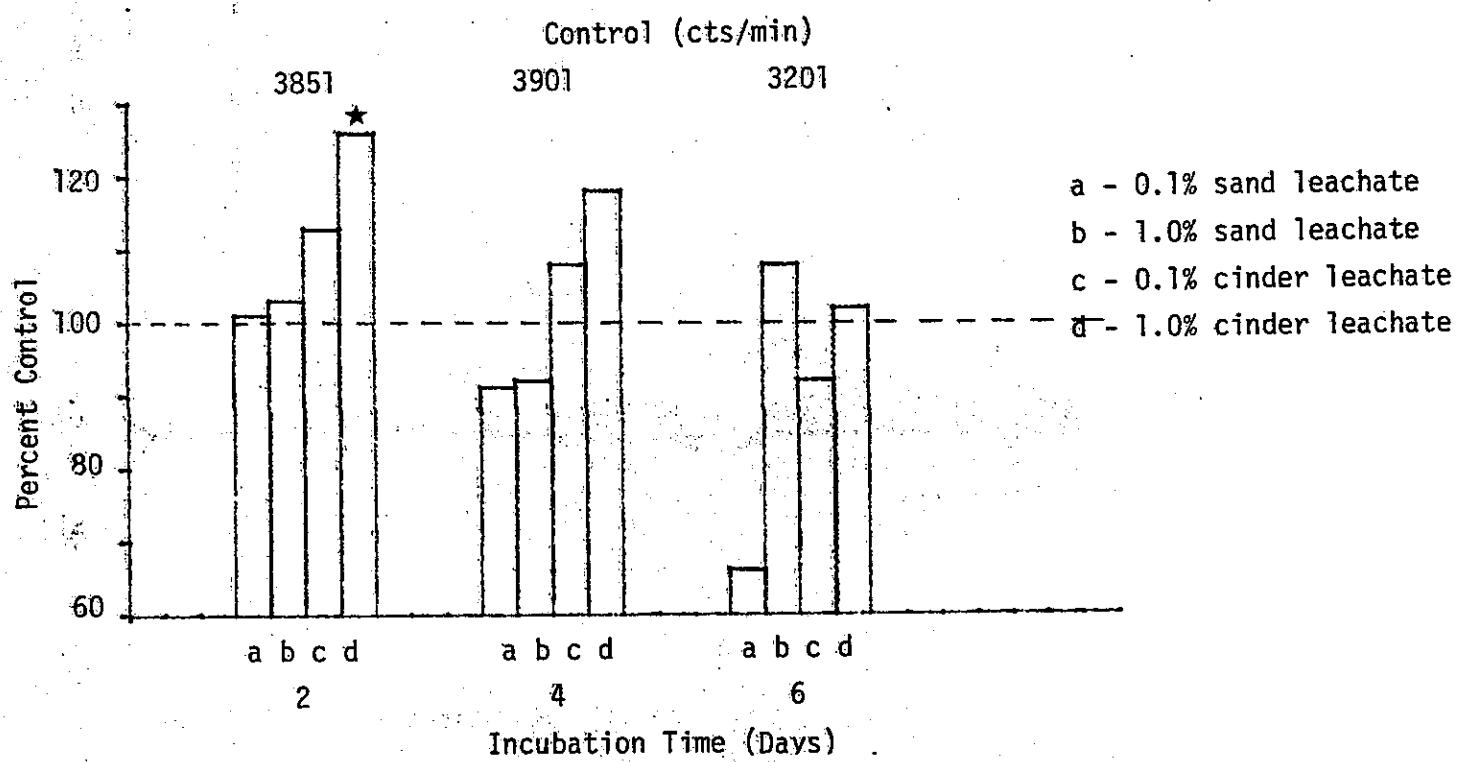


Figure 12. Bioassay of sand and cinder leachate additions to L. Tahoe water conducted 23-29 June 1981. (Bacterial)

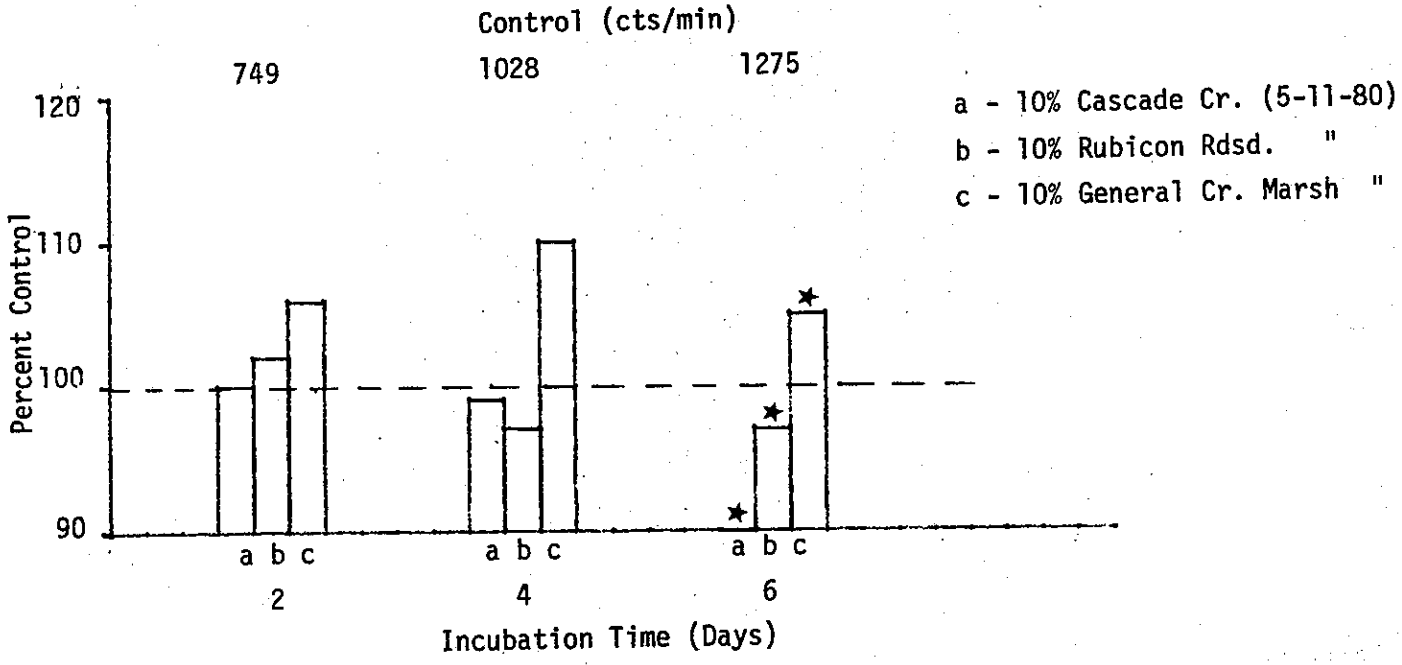


Figure 13. Bioassay conducted 10-16 December 1980. (Algal)

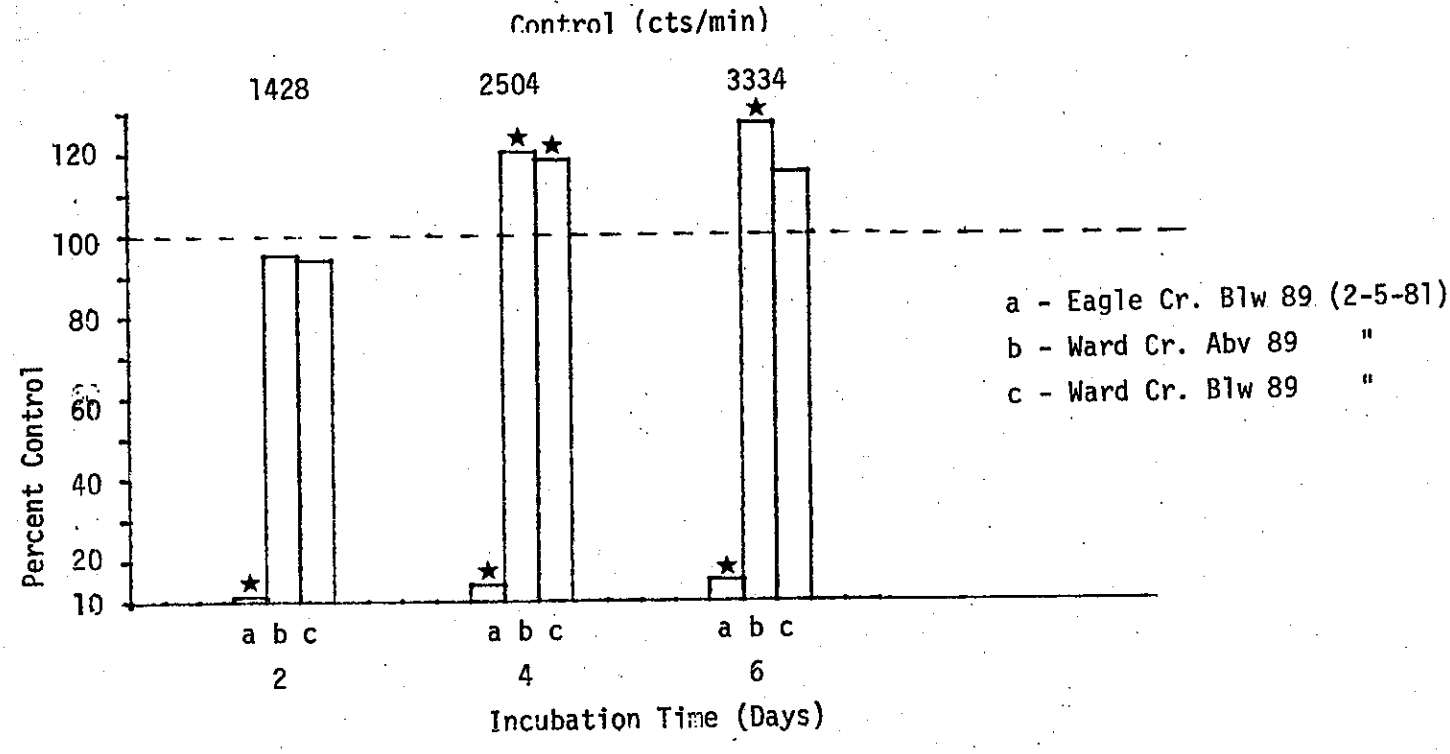


Figure 14. Bioassay conducted 7-13 February 1981. (Algal)

Appendix C: Mean ^{14}C Uptake and Periphyton Primary Production (s = 1 std. dev) (con't)

Bioassay No.	Treatment	Day 2		Day 4		Day 6	
		$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
9 (cont'd)	10 mg/l Processed	3208	209	3231	641	1438	204
	0.1% Sand Leachate	3905	586	3535	1061	2098	682
	1% Sand Leachate	3985	224	3587	556	3453	1289
	0.1% Cinder Leachate	4349	284	4219	1038	2941	276
	1% Cinder Leachate	4870	599	4605	827	3260	1043
10 (autotrophic) 5-11 July 81	Lake Tahoe @ Wallis Pier Control	402	21	781	26	1094	23
	10 mg/l Processed + "	432	36	729	40	1037	119
	Lake Tahoe @ Ward Cr. Control	431	36	954	82	1381	56
	10 mg/l Processed + "	402	31	993	83	1341	163
	Lake Tahoe @ Tallac Cr. Control	797	68	1801	100	2569	128
	10 mg/l Processed + "	802	53	1724	171	2639	148
	Lake Tahoe @ Snow Cr. Control	613	64	1020	58	1348	13
	10 mg/l Processed + "	551	41	1119	32	1447	103
11 (heterotrophic) 5-11 July 81	Lake Tahoe @ Wallis Pier Control	1862	636	3209	793	983	394
	10 mg/l Processed + "	2437	731	2804	919	3151	954
	Lake Tahoe @ Ward Cr. Control	4754	340	4129	1033	2114	491
	10 mg/l Processed + "	4244	826	3538	650	2864	900
	Lake Tahoe @ Tallac Cr. Control	7124	937	6988	650	5479	2548
	10 mg/l Processed + "	6309	1446	7652	595	7870	216
	Lake Tahoe @ Snow Cr. Control	4580	1184	5731	1391	4506	2522
	10 mg/l Processed + "	4450	387	5948	1607	6572	2116

12
(Periphyton)
7-8 July 1981

	PPr $mgC/m^2/hr$	Total Carbon gC/m^2	Specific Production mgC/g total C/hr
Control 1	13.78	16.11	0.86
	13.79	21.72	0.63
Control 2	12.38	16.01	0.77
	15.42	13.60	1.13
Processed Salt 1	9.72	16.45	0.59
1 mgCl/l	16.02	18.35	0.87
Processed Salt 2	5.01	12.02	0.42
1 mgCl/l	12.80	18.90	0.68
Processed Salt 1	12.11	15.07	0.80
10 mgCl/l	12.60	16.16	0.78
Processed Salt 2	9.60	18.34	0.52
10 mgCl/l	11.81	22.50	0.52
Kiln Dried Salt 1	8.60	10.55	0.82
1 mgCl/l	8.17	9.94	0.81
Kiln Dried Salt 2	9.61	12.69	0.76
1 mgCl/l	9.15	14.54	0.63
Kiln Dried Salt 1	9.28	13.47	0.69
10 mgCl/l	8.30	16.59	0.50
Kiln Dried Salt 2	7.05	14.80	0.48
10 mgCl/l	12.76	19.41	0.66

Appendix D: Bioassay Treatment vs. Control Contrasts.
Treatment Considered Not Significant (NS) at $p \geq .10$.

Bioassay No.	Treatment	Day 2	Day 4	Day 6	Day 8
1 (autotrophic)	0.1 mg/l Kiln Dried	.005	.025	.005	
	1 mg/l Kiln Dried	.010	.01	.025	
	10 mg/l Kiln Dried	.001	.025	.05	
	10% Cascade Cr.	NS	NS	.005	
	10% Hwy 89 Roadside	NS	NS	.05	
	10% General Cr. Marsh	NS	NS	.05	
2 (autotrophic	0.01 mg/l Kiln Dried	NS	NS	NS	
	0.1 mg/l Kiln Dried	NS	NS	NS	
	1 mg/l Kiln Dried	NS	NS	NS	
	10 mg/l Kiln Dried	NS	NS	NS	
	10% Eagle Cr. BLW 89	.001	.001	.001	
	10% Ward Cr. ABV 89	NS	.025	.025	
	10% Ward Cr. BLW 89	NS	.05	NS	
3 (autotrophic)	1 mg/l Reagent Grade	NS	NS	NS	NS
	10 mg/l Reagent Grade	NS	NS	NS	NS
	100 mg/l Reagent Grade	NS	NS	NS	NS
	1 mg/l Kiln Dried	NS	NS	NS	NS
	10 mg/l Kiln Dried	NS	NS	NS	NS
	100 mg/l Kiln Dried	NS	NS	NS	NS
	1000 mg/l Kiln Dried	.01	NS	NS	NS
	1 mg/l Processed	NS	NS	NS	NS
	10 mg/l Processed	NS	NS	.10	.10
	100 mg/l Processed	NS	NS	.10	.01
	10% Cascade Roadside Snow	NS	.025	.01	.001
	10% Ward Valley Snow	NS	NS	NS	NS
	10% Ward V. S. + 1 mg/l Kiln Dried	NS	NS	NS	NS
	10% Ward V. S. + 10 mg/l Kiln Dried	NS	NS	NS	NS
	10% Tahoe City Roadside Snow	NS	NS	.005	.001
	10% Eagle Cr. ABV 89	.05	NS	NS	NS
4 (autotrophic)	100 mg/l Kiln Dried	.001	.001	.010	.025
	1000 mg/l Kiln Dried	.001	.001	.001	.001
	100 mg/l Processed	.001	.100	NS	NS
	1000 mg/l Processed	.001	.001	.001	.001
	0.1% Cinder Leachate	NS	NS	.10	NS
	1% Cinder Leachate	.005	.001	.001	.001
	0.1% Sand Leachate	NS	NS	NS	NS
	1% Sand Leachate	NS	NS	NS	NS
	10% 1/5 Ward Bridge Seep.	.05	NS	.005	.001

Appendix D. (cont'd) Bioassay Treatment vs. Control Contrasts. Treatment Considered Not Significant (NS) at $p \geq .10$.

Bioassay No.	Treatment	Day 2	Day 4	Day 6
5 (heterotrophic)	1 mg/l Reagent Grade	NS	NS	NS
	10 mg/l Reagent Grade	NS	NS	NS
	100 mg/l Reagent Grade	NS	NS	NS
	1 mg/l Kiln Dried	NS	NS	NS
	10 mg/l Kiln Dried	NS	NS	NS
	100 mg/l Kiln Dried	NS	NS	NS
	1000 mg/l Kiln Dried	NS	.10	.001
	1 mg/l Processed	NS	NS	NS
	10 mg/l Processed	NS	NS	NS
	100 mg/l Processed	NS	NS	NS
	1000 mg/l Processed	.005	.025	NS
6 (autotrophic)	1 mg/l Kiln Dried	NS	NS	NS
	10 mg/l Kiln Dried	NS	NS	NS
	50 mg/l Kiln Dried	.005	NS	NS
	100 mg/l Kiln Dried	.005	.05	.025
	1 mg/l Processed	.025	NS	NS
	10 mg/l Processed	.05	NS	.005
	50 mg/l Processed	.01	NS	.005
	100 mg/l Processed	.005	NS	NS
7 (heterotrophic)	1 mg/l Kiln Dried	NS	NS	NS
	10 mg/l Kiln Dried	NS	NS	NS
	50 mg/l Kiln Dried	NS	NS	NS
	100 mg/l Kiln Dried	NS	NS	NS
	1 mg/l Processed	NS	NS	NS
	10 mg/l Processed	NS	NS	NS
	50 mg/l Processed	NS	NS	NS
	100 mg/l Processed	NS	NS	NS
8 (autotrophic)	1 mg/l Kiln Dried	NS	NS	NS
	10 mg/l Kiln Dried	NS	NS	NS
	1 mg/l Processed	.025	NS	NS
	10 mg/l Processed	NS	NS	NS
	0.1% Sand Leachate	.01	NS	NS
	1% Sand Leachate	NS	NS	NS
	0.1% Cinder Leachate	NS	NS	NS
	1% Cinder Leachate	NS	NS	NS
9 (heterotrophic)	1 mg/l Kiln Dried	NS	NS	NS
	10 mg/l Kiln Dried	NS	NS	NS
	1 mg/l Processed	NS	NS	.10
	10 mg/l Processed	.05	NS	.025
	0.1% Sand Leachate	NS	NS	NS
	1% Sand Leachate	NS	NS	NS
	0.1% Cinder Leachate	NS	NS	NS
	1% Cinder Leachate	.01	NS	NS

Appendix D. (cont'd) Bioassay Treatment vs. Control Contrasts. Treatment Considered Not Significant (NS) at $p \geq .10$.

Bioassay No.	Treatment	Day 2	Day 4	Day 6
10 (autotrophic)	10 mg/l Processed to W.P.	NS	NS	NS
	10 mg/l Processed to W.C.	NS	NS	NS
	10 mg/l Processed to T.C.	NS	NS	NS
	10 mg/l Processed to S.C.	NS	NS	NS
11 (heterotrophic)	10 mg/l Processed to W.P.	NS	NS	NS
	10 mg/l Processed to W.C.	NS	NS	NS
	10 mg/l Processed to T.C.	NS	NS	.10
	10 mg/l Processed to S.C.	NS	NS	NS
12 (periphyton)		- 24 hr -		
	1 mg/l Kiln Dried	NS		
	10 mg/l Kiln Dried	NS		
	1 mg/l Processed	NS		
	10 mg/l Processed	NS		

Appendix E: Chloride Analyses

Station	Date	Chloride (mg/l)
Lake Tahoe (So. of Ward Cr)	12-07-80	2.218
	2-05-81	2.127
$\bar{x}$ = 2.054	3-07-81	2.314
	4-02-81	2.127
s = .166	5-03-81	1.874
	5-30-81	1.963
	6-23-81	1.931
	7-05-81	1.874
Lake Tahoe (Ward Cr inlet)	7-05-81	1.867
Lake Tahoe (Snow Cr inlet)	7-05-81	2.100
Lake Tahoe (Tallac Cr inlet)	7-05-81	2.056
Blackwood Cr (Highway 89)	4-02-81	.382
	5-03-81	.230
Cascade Cr (Highway 89)	5-11-81	.248*
	5-11-81	.261
	2-28-81	.964
Dollar Cr (Highway 28)	4-02-81	.897
Eagle Cr (Highway 89)	2-05-81	.366*
	2-05-81	.245
General Cr (Highway 89)	5-11-80	.313*
	5-11-80	.296
	4-06-81	.316
$\bar{x}$ = .255	4-30-81	.233
	5-03-81	.240*
s - .036	5-03-81	.256
	5-07-81	.224
	5-13-81	.224
	5-18-81	.264
	5-26-81	.224
General Cr Marsh (Highway 89)	5-11-80	.343
Incline Cr (Highway 28)	4-20-81	4.692
	5-03-81	1.751
	5-09-81	1.362
$\bar{x}$ - 2.291	5-20-81	2.333
	6-01-81	2.070
s = 1.228	6-17-81	1.539

* sampled above main highway

Station	Date	Chloride (mg/l)
Meeks Cr (Highway 89)	4-02-81	.452
	5-03-81	.212
Snow Cr (Highway 28)	5-03-81	9.150
	5-05-81	7.710
$\bar{x}$ = 7.827	5-08-81	7.127
	5-11-81	9.070
s = 2.387	5-14-81	8.500
	5-18-81	10.290
	7-05-81	2.941*
Tallac Cr (Highway 89)	7-05-81	.183
Third Cr (Highway 28)	5-03-81	.942
	5-11-81	.964
$\bar{x}$ = 1.281	5-20-81	2.101
	6-01-81	1.229
s = .424	6-10-81	1.200
	6-17-81	1.252
Trout Cr (Highway 50)	3-11-81	1.182*
	4-22-81	.697*
$\bar{x}$ = .675	4-30-81	.561*
	5-09-81	.592*
s = .262	5-19-81	.583*
	5-30-81	.434*
Upper Truckee R (Highway 50)	4-22-81	2.722
	4-28-81	1.668
$\bar{x}$ = 1.624	5-03-81	1.331
	5-12-81	.933
s = .684	5-19-81	2.073
	5-26-81	1.018
Ward Cr (Highway 89)	2-05-81	.534*
	2-05-81	.512
$\bar{x}$ = .262	4-02-81	.606*
	4-02-81	.594
s = .159	4-19-81	.251
	4-30-81	.198
	5-02-81	.172
	5-03-81	.309*
	5-03-81	.256
	5-09-81	.146
	5-16-81	.152
	5-18-81	.156
	7-05-81	.187
Residential Drainage Tahoma (Highway 89)	2-05-81	6.095*
	2-05-81	6.974

* sampled above main highway

Station	Date	Chloride (mg/l)
Ward Valley Snow (Twin Pks Rd)	2-28-81	.382
	4-02-81	.250
Cascade Creek Snow (@ Highway 89)	2-28-81	1.214
Tahoe City Roadside Snow (@ Highway 89)	2-28-81	250.461
Culvert Collecting r/off from above	2-28-81	41.127
Roadside Snow Melt (Rubicon area)	5-11-80	8.555

* Sampled above main highway.

Appendix F: Phytoplankton Identification and Enumeration

Bioassay 3	Initial	Final Control	Final 100 ppm Kiln	Final 100 ppm Proc.
	Cells ml^{-1}			
Cyclotella ocellata	3	38	24	8
Stephanodiscus alpinus	43	40	19	20
Asterionella formosa	391	710	673	865
Synedra radians	13	31	27	42
Kephyrion c.f. rubri-claustri	85	160	155	287
Salpinogoeca frequentissima	123	26	34	96
Monoraphidium contortum	50	35	17	37
Tetraedron minimum var. tetralobulatum	18	28	5	14
microflagellate "A"	28	31	24	14
(3μ) Microflagellate	24	78	165	96
LRGT's	333	1264	1032	1161

Bioassay 6	Initial	Final Control	Final 10 ppm Kilm	Final 100 ppm Kiln	Final 100 ppm Proc.
Cyclotella ocellata	8	11	10	4	12
Synedra Radians	10	14	13	21	20
Pseudokephyrion ovum	69	79	39	41	49
Chrysolykos planctonicus	10	55	21	17	55
Dinobryon c.f. divergens	5	38	31	6	12
Unidentified Chyrosophyte	16	107	83	43	29
(3μ Microflagellate	410	927	775	627	1075
"Interfilum-like"	21	22	44	33	26
LRGT's	39	3	10	4	14